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CONTENTS

The stimulatory effect of light upon growth and CO_2 fixation in Blastocladiella. I. The S. K. I. cycle
EDWARD C. CANTINO AND E. A. HORENSTEIN 777

In vitro effect of mycothricin on plant pathogenic bacteria and fungi.....G. RANGASWAMI 800

Observations on Gymnoascaceae. III. Developmental morphology of Gymnoascus reessii, a new species of Gymnoascus and Eidamella reflexa.....HAROLD H. KUEHN 805

A preliminary survey of the genus Platygloea
ROBERT J. BANDONI 821

Marine Fungi. II. Ascomycetes and Deuteromycetes from submerged wood.....T. W. JOHNSON, JR. 841

The Pleurotus-hirtus-complex.....ROLF SINGER 852

A new genus of downy mildew on sorghum
P. N. RAO, M. A. SALAM AND M. J. THIRUMALACHAR 860

The genus Retinocyclus
J. WALTON GROVES AND DOREEN E. WELLS 865

The Ustilaginales of Bihar. II. Five undescribed species
J. N. MISHRA 872

Notes and Brief Articles..... 877

Lange: *Picos;* Kennedy: *Dacrymyces;* Plakidas: *Cercospora;* Goos: *Melanospora;* Schatz et al.: Amino acids; Mycological program at New York; Covert: Colony mounts; Stain certification; Financial Report.

Index..... 888

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MYCOLOGIA

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VOL. XLVIII NOVEMBER–DECEMBER, 1956 NO. 6

THE STIMULATORY EFFECT OF LIGHT UPON GROWTH AND CO_2 FIXATION IN BLASTOCLADIELLA. I. THE S. K. I. CYCLE[1]

EDWARD C. CANTINO[2] AND E. A. HORENSTEIN

(WITH 3 FIGURES)

Current knowledge of the relation between metabolism and morphogenesis in the aquatic fungus *Blastocladiella emersonii* is summarized in recent progress reports (**4**, **5**, **6**).[3] Investigations of a spontaneous mutant of *B. emersonii* (hereafter called BEM), bright orange in color because of its content of γ-carotene (**8**, **9**), have also played a significant role in our understanding of morphogenetic mechanisms in *Blastocladiella*. We have now observed that both mutant and wild type proliferate more rapidly in the light than in the dark. Insofar as these organisms bear carotenoids but are apparently free of chlorophyll, this

[1] The research on the mutant strain of *Blastocladiella* was carried on at the University of Pennsylvania and was supported by grant E-581 (C3) from the Division of Research Grants of the National Institute of Health, Education, and Welfare. We wish to thank Dr. W. Stepka and Mrs. Selma Brodsky for their help in initiating some of this work. Most of the study with the wild type *B. emersonii* was performed at the Brookhaven National Laboratory where it also was supported, in part, by the above grant E-581 (C3). We are indebted to Dr. H. Curtis for making available the facilities of the Biology Department, Brookhaven National Laboratory, and to Dr. Martin Gibbs for his interest in our project. We are particularly grateful to Dr. R. C. Fuller, in whose laboratory we worked, for his generosity in providing labeled bicarbonate, space, and equipment, and his advice and cooperation during the course of our studies.

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[3] See also Cantino, E. C., and E. A. Horenstein. Gamma and the cytoplasmic control of differentiation in *Blastocladiella*. Mycologia **48**: 443–446. 1956.

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discovery appears to be of considerable significance. We therefore wish to report upon our observations.

MATERIALS AND METHODS

Liquid cultures of thin-walled plants of *B. emersonii* were grown at 23° C on medium PYG (Difco peptone, 1.25 g, Difco yeast extract, 1.25 g, glucose, 3.0 g, bromcresol purple, 0.6 ml of 0.4% aq solution, and water to make one liter) and neutralized intermittently (**8**, **9**). Cultures of resistant sporangial plants were grown at 23° C as described by Cantino and Horenstein (**7**) on medium PYG$\frac{1}{2}$B (medium PYG containing 10^{-2} M bicarbonate).

The mutant, BEM, was that first described by Cantino and Hyatt (**8**, **9**), cultures of which must be routinely subcultured every two weeks at 20° C on solid medium PYG. For experimental purposes, liquid cultures were also grown on medium PYG and neutralized intermittently.

In order to compare the growth of liquid cultures of *B. emersonii* in light and darkness, some culture vessels (500 ml Erlenmeyer flasks) were covered with aluminum foil while others were exposed to direct, continuous illumination from below with 40-watt fluorescent lights. The light path was 3 cm and yielded 60–80 foot candles at the lower surface of the culture. Forced circulation of air between vessels and light source maintained a temperature of 22° to 24° during growth. Except for a few unaerated controls, cultures were aerated through fritted glass dispersion tubes.

In order to compare the growth of cultures of the mutant in light and darkness, Erlenmeyer flasks were painted black but the bottoms were left clear. Thus, light-grown cultures were illuminated from below; for dark-grown cultures, flask bottoms were covered with aluminum foil. The light source was a pair of adjacent fluorescent lights (either 15 or 40 watts). Cultures were incubated directly over the lights, either with a 1 cm light path through a 0.5 cm clear glass filter or with a 5 cm light path through a 2 cm water filter.

Inocula of spore suspensions of *B. emersonii* were prepared according to Cantino and Horenstein (**7**). Inocula of the mutant were prepared by cutting out colonies derived from one- or two-week-old cultures on agar media and placing them in 5 ml of distilled water. Thousands of swarmers were discharged within 4 to 6 hours, and uniform aliquots of these suspensions were used to inoculate all culture vessels in any one experiment.

Dry weights were determined by harvesting on filter paper, washing with water, and drying to constant weight at 90° C. Carotenoids were

extracted with acetone and petroleum ether (8). Extracts were dried with anhydrous sodium sulfate, and their transmission was compared with standard curves for γ-carotene in a Klett-Summerson colorimeter (green filter).

Studies of the effect of light upon the carbon metabolism of preformed cell suspensions and cell-free homogenates were made at both 23° and 26–27° C, respectively, in glass-stoppered, 250 ml Erlenmeyer flasks sealed with silicone grease. These vessels were placed on a rotary shaker (200 rpm) under a bank of eight 40-watt fluorescent lights. The light path was 44 cm and yielded about 750 foot candles at the surface of the reaction mixture. For the isolation of labeled intermediates, cells were extracted with hot 80% ethyl alcohol (50 ml per 175 mg wet weight of fungus), and washed with 2 ml aliquots of 20% alcohol. Extracts were concentrated to 3 ml by vacuum distillation at 30° C and then evaporated to dryness in a vacuum dessicator. The residue was dissolved in 2 ml of 20% alcohol, and this solution was used for subsequent analyses.

Two-dimensional chromatography and radioautography were carried out as described by Benson *et al.* (2). The solvent system employed for *B. emersonii* was phenol and butanol-propionic acid (2); that for the mutant was phenol and butanol-acetic acid. One-dimensional chromatography with butanol-formic acid followed the procedures of Buch *et al.* (3) and Lugg and Overell (12) as modified slightly by Cantino and Horenstein (7). Deproteinization and clearing of reaction mixtures and extracts were accomplished with either zinc hydroxide (13) or ethyl alcohol. When labeled acidic compounds were to be co-chromatographed, three different solvents were usually employed: butanol-formic acid, butanol-propionic acid, and phenol.

Assays for oxidative activity in homogenates of freshly-harvested plants were those described by Cantino and Hyatt (8, 9). Dried powdered mats of the fungus were prepared, stored, and assayed for enzymatic activity as described by Cantino and Horenstein (7) and Cantino and Hyatt (9).

In order to establish the effect of light upon the enzymatic activity of cell-free preparations in experiments not involving C^{14}, reaction mixtures were incubated and agitated (ca 100 rpm) in 15 ml Warburg vessels in a constant temperature bath at 29° C. Either vessels were covered with foil or they were illuminated from below through a 2 cm light path with a 40-watt fluorescent lamp.

Glucose analyses were made according to Somogyi (16) and Nelson (14) as outlined by Neish (13). Lactic acid was determined according

to Fridemann and Graeser (11). All other specific details will be found in the context and figure legends.

EXPERIMENTS WITH THE MUTANT, BEM

THE EFFECT OF LIGHT ON GROWTH OF BEM. For these studies, it was imperative to determine the Q_{10} for growth. BEM was grown in the dark without shaking at 20° C and 30° C and harvested after 5 days. The Q_{10} for both dry weight and total acid production during growth was 1.5.

Growth in the dark at 30° C was then compared with that under continuous illumination at 30° C. No fluctuations in temperature greater than 0.5° C were detected between illuminated and non-illuminated cultures. However, illuminated cultures produced far

TABLE I

YIELD FROM CULTURES OF BEM GROWN AT CA 30°C ON MEDIUM PYG

Age of culture	Volume of cultures	Aerated?	Average dry weight (mg) per culture, when grown:		% increase in yield due to illumination
			with illumination	without illumination	
10	500 ml	yes	395	246	60
10	75 ml	no	160	120	33
13	75 ml	no	205	85	141
7	2000 ml	yes	481	317	52

greater yields than dark-grown culture (TABLE I). Visual observations suggested that the stimulation by light was greatest in the early stages of growth; these impressions were corroborated by subsequent analyses (see later).

THE RELATION BETWEEN SYNTHESIS OF CAROTENOID AND TOTAL DRY WEIGHT OF BEM. The visible coloring matter in BEM appeared to be almost exclusively γ-carotene (8). Because carotenoid might have mediated the stimulatory effect of light described above, the relation between growth and synthesis of carotene was established. Non-shaken, 150 ml cultures were incubated at 20° C in the dark in 250 ml Erlenmeyer flasks and harvested at intervals. Carotenoid was not detected until one-third of the growth period had been completed, following which it accumulated at a rapidly accelerating pace (FIG. 1f). It is interesting that during the generation time of a single orange ("male") plant of *B. emersonii*, too, visible carotenoid is not laid down until the

plant is well along in ontogeny; then, the full complement of pigment is synthesized in a matter of minutes (**10**).

Accumulation of carotene, however, was greatly influenced by environmental conditions. For instance, it was evident that cultures grown at both 20° C and 30° C in the dark were much more orange in color than those grown under identical conditions but exposed to continuous illumination. Actually, the content of γ-carotene in full-grown cultures incubated in the dark varied from 0.3 to 0.5 μg per mg dry weight; in the light, it was only about 0.1 μg per mg dry weight. Whether or not this was due to differential rates of synthesis of carotenoid, photodestruction of carotenoid, or a combination of the two remains to be determined. From the start, however, it seemed likely that carotene did not mediate the stimulatory effect of illumination; light had produced its greatest effect during the first half of the growth period, when the carotenoid content of cultures was negligible.

THE EFFECT OF BICARBONATE UPON GROWTH OF BEM. Because of our studies of the effect of bicarbonate on morphogenesis (**4**), we wondered if the increased growth in illuminated cultures might have been due to increased rates of CO_2-fixation. To test this, BEM was grown in illuminated vessels at ca 27° C. One half of them contained 6.6×10^{-4} M bicarbonate while the other half was adjusted to the same pH with NaOH. Additional bicarbonate (100 μM per 150 ml) was added to the first half of the set after 2, 4, and 7 days. Cultures were maintained at a relatively constant pH by addition of sterile alkali whenever the indicator in the medium revealed a color change. Non-illuminated cultures were also grown in the presence and absence of bicarbonate. At intervals, cultures were harvested in triplicate and their dry weights determined. During the first 4 or 5 days of growth in the light, cultures incubated in the presence of bicarbonate yielded from 100 to 150% more plant material than those incubated in the absence of bicarbonate. In the dark, however, bicarbonate yielded only a 25 to 50% increase. Following the first 4 to 5 day incubation period, rates of growth in the presence and absence of bicarbonate became roughly comparable. Thus, bicarbonate had a much greater stimulatory effect upon proliferation of illuminated cultures during the early stages of growth than it did upon the non-illuminated ones.

Acidic, metabolic products recovered from the spent culture media were tentatively identified by chromatography and subsequent co-chromatography with authentic samples, and direct chemical analyses (see MATERIALS AND METHOD). They consisted mainly of lactic acid and rather small quantities of isocitric, α-ketoglutaric, succinic and fumaric

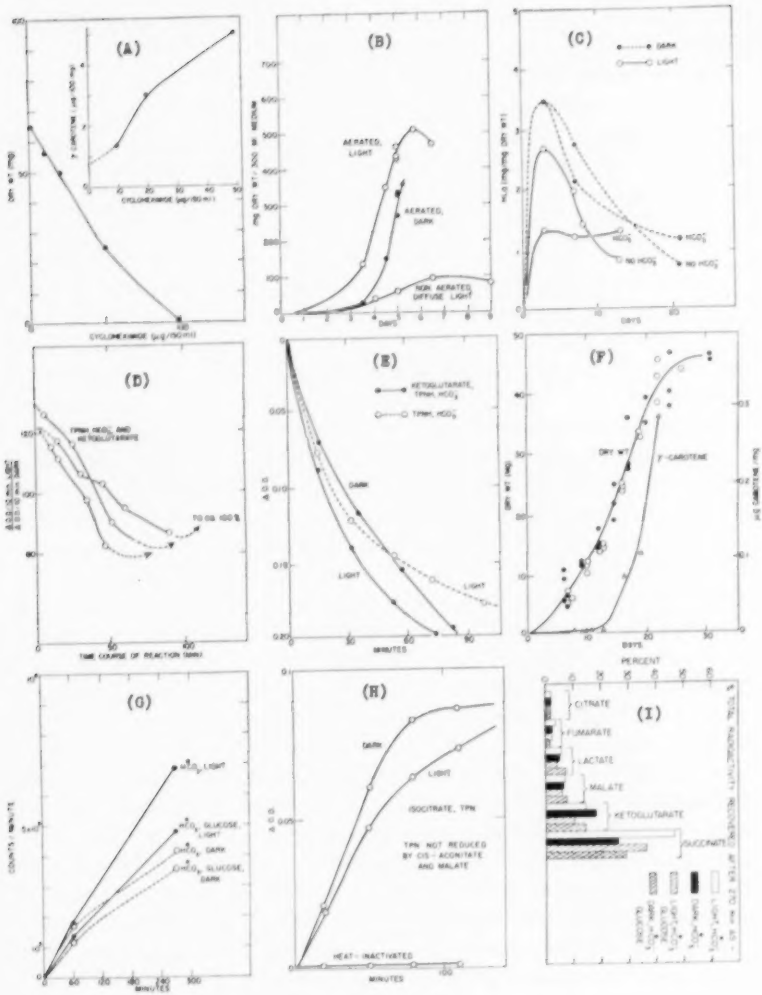

FIG. 1, A–I.

FIG. 1. A. The growth of *B. emersonii* without forced aeration in 150 ml of medium PYG containing different concentrations of cycloheximide ("Actidione," Upjohn and Co.). Cultures were harvested after 11 days of growth at 25° C. A, insert. The production of γ-carotene during the growth of *B. emersonii* on a rotary shaker (Ross-Kershaw Type; 180 oscillations/minute) in 150 ml of medium PYG½B containing different concentrations of cycloheximide. Cultures were harvested after 10 days of growth at 25° C. B. The growth of *B. emersonii* at 32° C

acids. Lactate determinations on filtrates from illuminated cultures revealed (FIG. 1c) that bicarbonate depressed the rate of lactate production per unit weight of organism during the early stages of growth. However, bicarbonate did not depress the rate of lactate production in the non-illuminated cultures!

CO_2 CONSUMPTION BY ILLUMINATED AND NON-ILLUMINATED CULTURES OF BEM. In the foregoing experiments, (1) light stimulated growth of BEM, and (2) bicarbonate further accelerated growth and depressed production of lactic acid provided cultures were illuminated. These observations provided some evidence for our notion that CO_2 fixation might be involved in the stimulatory effect of light upon growth. This idea was then put to a more direct test. BEM was grown in 2000 ml of medium PYG containing 10^{-4} M bicarbonate in illuminated and non-illuminated flasks, 2.0 ml of 10^{-1} M bicarbonate were added to each flask on the 3rd, 4th, and 5th days after inoculation, and cultures were neutralized periodically with NaOH to maintain a constant pH. During growth, they were aerated with CO_2-free air, and the effluent

in 300 ml of medium PYG under different conditions of aeration and illumination. C. The production of lactic acid during the growth of illuminated and non-illuminated cultures of BEM in the presence and absence of bicarbonate (*cf.* context for details). D. Results of 3 different experiments illustrating the relative rates of oxidation of reduced TPN in the light as compared to those in the dark at different stages during the reaction. Legend for vertical axis, $\times 100$. Reaction mixtures were prepared as in E. E. The oxidation of reduced TPN (as measured by its oxidation at 340 mμ) by illuminated and non-illuminated supernatants prepared as in H. Reaction mixtures contained: 10^{-3} M α-ketoglutarate (when indicated in graph), 0.8 ml; reduced TPN (1.0 mg/4.0 ml), 1.0 ml; supernatant, 1.3 ml and 10^{-1} M bicarbonate, 0.8 ml (*cf.* context for further details). F. The growth of, and synthesis of γ-carotene by, 150 ml, unaerated, non-illuminated cultures of BEM at 20° C. Solid and hollow circles represent results from 2 different experiments. G. The fixation of labeled bicarbonate by non-proliferating suspensions of *B. emersonii* incubated with and without illumination, and in the presence and absence of unlabeled glucose. Data are expressed in terms of total counts/minute recovered after the removal of unassimilated bicarbonate (*cf.* context for further details). H. The oxidation of isocitrate (as measured by the reduction of TPN at 340 mμ) by illuminated and non-illuminated supernatants. Supernatants were prepared by homogenizing 150 mg of 9-month-old, dried, resistant sporangia of *B. emersonii* in 30 ml M/7.5 phosphate buffer, pH 6.8 for 1 hour in an ice bath and then centrifuging at $500 \times G$ for 5 minutes. Reaction mixtures contained: 2×10^{-2} M isocitrate, 0.8 ml; TPN (1.0 mg/4.0 ml), 1.0 ml; supernatant, 1.3 ml; and water to make 3.9 ml total volume (*cf.* context for further details). I. Recovery of labeled compounds resulting from fixation of labeled bicarbonate by illuminated and non-illuminated suspensions of *B. emersonii* incubated in the presence and absence of unlabeled glucose. Oxalate recoveries, amounting to about 5% of the total activity, are not shown in the histogram (*cf.* context for further details).

gas was trapped in standard alkali. After 6 days, cultures were acidified and aerated vigorously for 2 hours to collect all the remaining CO_2 in the system. Final dry weights were 481.9 mg for illuminated cultures and 316.5 mg for non-illuminated cultures. The quantity of CO_2 recovered in the dark was almost twice that in the light—16.8 μM per mg dry weight for illuminated cultures, and 29.1 μM per mg for non-illuminated ones. If it is assumed that the rate of respiration was not affected by the presence or absence of light, the illuminated plants must have fixed an additional 12.3 μM, or 541 μg, of CO_2 per mg dry weight over the 6-day growth period. Thus, the additional CO_2 fixed in the light could have accounted for much of the increased growth of illuminated cultures of BEM.

STUDIES WITH LABELED CO_2 AND NON-PROLIFERATING PLANTS OF BEM. Having established in various ways that illumination affected the growth and metabolism of proliferating plants of BEM, 12-day-old cultures were harvested, washed, and suspended in water. Labeled bicarbonate (0.25 ml of a solution containing 58μc/ml, plus unlabeled bicarbonate to yield a final concentration of 10^{-4} M) was added to each suspension; one was illuminated for 90 minutes while the other was kept in the dark. The fungus was then extracted with hot 80% ethyl alcohol, washed successively with 4 aliquots of 30% alcohol, and the combined extracts were concentrated by vacuum distillation. Aliquots of this extract were counted directly while others were chromatographed with phenol and butanol-acetic acid (**17**). Only about 1–2% of the labeled carbon was fixed into water-soluble compounds in the 90 minutes of incubation; however, illuminated cultures fixed 13% more carbon dioxide than did non-illuminated ones. In both cases, following resolution of the labeled constituents by chromatography, about 80% of the radioactivity was recovered in compounds tentatively identified by position alone as lactic, malic, succinic, isocitric, glutamic, and aspartic acids. The succinic acid was then identified by co-chromatography with authentic samples. With the exception of succinate, the %-recovery for the other five acids was of the same order of magnitude in the light and in the dark. However, the labeled succinate accounted for only 13.3% of the total activity in the dark, but 25.2% in the light. The label did not appear in an easily-detectable pool of other amino acid constituents of BEM which, by position and color with ninhydrin alone, were tentatively characterized as leucine, isoleucine, phenylalanine, valine, methionine, tyrosine, proline, asparagine, and an unusually high concentration of hydroxyproline.

Once again, but this time with pre-formed, non-proliferating plants,

illumination had induced an increased fixation of carbon dioxide by BEM. There was no qualitative difference in the distribution of labeled compounds for illuminated and non-illuminated cell suspensions. But, there was a quantitative difference; recovery of labeled succinate was much higher in the light than in the dark.

EXPERIMENTS WITH B. EMERSONII

THE EFFECT OF LIGHT ON GROWTH OF B. EMERSONII. Preliminary observations revealed that when 300 ml cultures of *B. emersonii* were grown in the light and dark, and then harvested after ca 4 days, the yield (dry weight) in the light was always 150 to 350 mg whereas that in the dark varied between zero and 100 mg. Subsequently, growth curves were established. Vessels containing 300 ml of medium PYG were inoculated with uniform aliquots from a single suspension of swarmers of *B. emersonii*, incubated in the light and in the dark, aerated continuously through fritted glass dispersion tubes, and harvested at intervals. Controls without aeration and subjected only to diffuse light from open windows during the day were also inoculated at the same time. Dry weight determinations (FIG. 1b) showed: (1) that aerated cultures grew 6 to 8 times more rapidly than non-aerated ones, and (2) that during the first half of the growth period, illumination had a stimulatory effect upon the rate of growth of aerated cultures. Once again, the temperature of illuminated cultures did not fluctuate by more than 0.5° C from that of non-illuminated ones, and the Q_{10} for growth was about 1.5. Thus, the increased growth in the light was attributed to a direct effect of illumination.

THE CAROTENOIDS IN B. EMERSONII. Although the mutant strain contained γ-carotene (**8**; also, experiments already described), we could not detect carotenoids in *B. emersonii* grown on medium PYG as described above. And yet, the fact that illumination accelerated growth demanded the presence of some sort of a light receptor in the fungus. Therefore, this aspect of the problem was pursued further. It was known that *Blastocladiella* could develop along an alternate morphogenetic pathway which led to the production of distinctly different, brown, thick-walled, pitted, non-proliferating, resistant sporangial plants instead of the aforementioned thin-walled, colorless plants. These resistant sporangial plants are not formed during active proliferation in liquid cultures except at the end of the growth period, but they can be induced to form from the very start if bicarbonate is incorporated in the medium (**7**, and references therein). To complete the survey,

therefore, resistant sporangial plants were also analyzed for carotenoids; the results revealed that they did indeed contain carotene, but at a much lower level (ca 0.01 μg per mg dry weight) than that found in the mutant strain of *Blastocladiella*. The absorption data on extracts of the pigment were identical with those (**8**) for BEM, and suggested once again that γ-carotene was practically the only carotenoid present. Thus, for the wild type *B. emersonii*, it was necessary to conclude: first, that carotenoid synthesis was associated only with the alternate developmental pathway leading to formation of non-proliferating resistant sporangial plants; and second, that it was not associated with the thin-walled, colorless plants found in the proliferating cultures which grew more rapidly in the light than in the dark.

Finally, we tried to provide some assurance that the apparent lack of carotenoids in colorless, thin-walled plants was not simply a reflection of a very low concentration of carotene, not detectable by our methods. Attempts were made to induce increased synthesis of carotenoid in liquid cultures of both the resistant sporangial plants and the thin-walled plants by growing them in the presence of cycloheximide. This was done because cycloheximide had induced a significant increase in the incidence of orange ("male") plants in populations of *B. emersonii* grown on agar media (**10**).

Cycloheximide did increase the γ-carotene content of resistant sporangial plants (insert, FIG. 1a). However, although this antibiotic also led to the expected decreased rates of growth of thin-walled plants (FIG. 1a), carotenoid was still not detected. Once again, it appeared as if carotenoid synthesis was associated only with the developmental path leading to resistant sporangial plants which matured and then ceased growth, and not with that leading to the actively-proliferating, thin-walled plants whose growth was accelerated by light. Therefore, further studies with cycloheximide were postponed, and it was concluded that the stimulatory effect of light was probably not mediated by γ-carotene, but rather by some other substance or substances.

THE UPTAKE AND SUBSEQUENT DISTRIBUTION OF LABELED CARBON COMPOUNDS BY B. EMERSONII. Our notions about the biochemical basis for morphogenesis in *Blastocladiella* have been based, in large measure, upon the belief that the oxidative decarboxylations mediated by isocitric dehydrogenase and ketoglutarate oxidase were CO_2-sensitive loci; that is, loci at which increased concentrations of bicarbonate decreased the rate of, brought to a standstill, and then even reversed part of the tricarboxylic acid cycle (**4**, **6**, **7**, **9**). Therefore, it was assumed as a working hypothesis: first, that the increased growth of illuminated plants

of *B. emersonii* might have been due to a light-stimulated fixation of CO_2 at the aforementioned sites with a concomitant accumulation of C_6 and/or C_4 acids such as succinate; and second, that if this were so, the presence of glucose might represent a potential force which would tend to drive the tricarboxylic acid cycle in its normal direction and thus tend to impede the fixation of CO_2 at these sites in the cycle. Using labeled bicarbonate and labeled glucose, attempts were made to establish whether or not these suggestions were correct.

Uptake and distribution of labeled bicarbonate and labeled glucose by pre-formed plants of B. emersonii. In order to ascertain what quantities of labeled compounds should be used in these experiments, 9-day-old, non-shaken cultures of *B. emersonii* were harvested and washed thoroughly with water. The fungus was resuspended in a series of vessels containing 2×10^{-3} M glucose, pH 8.5, and shaken at 23° C (175 mg wet weight per 10 ml total volume). The plant material was removed by filtration after 10, 30, 60, 120, and 270 minutes, and filtrates were analyzed for residual glucose. The uptake pattern was roughly linear; after 270 minutes of incubation, 8.5 μM, or 43% of the original 20 μM, of glucose had been consumed. Similar experiments, using 100 mg instead of 175 mg of cells and 100 μM of glucose, yielded similar uptake curves, except that about 25 μM of the original 100 μM of glucose were utilized after 270 minutes.

Four different 10 ml reaction mixtures were then prepared in quadruplicate as follows: (1) uniformly-labeled glucose, 20 μM, contaminated with 5% uniformly-labeled fructose (specific activity, 0.53 μc per mg); (2) labeled glucose as in (1), and unlabeled bicarbonate, 100 μM; (3) labeled bicarbonate, 12.2 μM (specific activity, 18 μc per μM) and unlabeled bicarbonate, 87.8 μM; (4) bicarbonate as in (3) plus unlabeled glucose, 20 μM. Pre-formed plants of *Blastocladiella* (175 mg wet weight) were incubated in each mixture in the light and dark. After 60 and 270 minutes, the fungus was extracted with hot alcohol (*cf.* MATERIALS AND METHODS). Fifty and 500 μl of the extracts were acidified with acetic acid to release residual bicarbonate and then counted directly on planchets for total radioactivity; chromatograms and radioautographs were prepared from comparable aliquots. Prior to chromatography, the origins were counted directly with an end-window Geiger tube.

Analysis of the series containing labeled glucose and unlabeled bicarbonate revealed that: (1) only the glucose, and not the fructose contaminant, was consumed by *Blastocladiella;* (2) plants incubated in the light for 270 minutes utilized from 30 to 50% more glucose than those

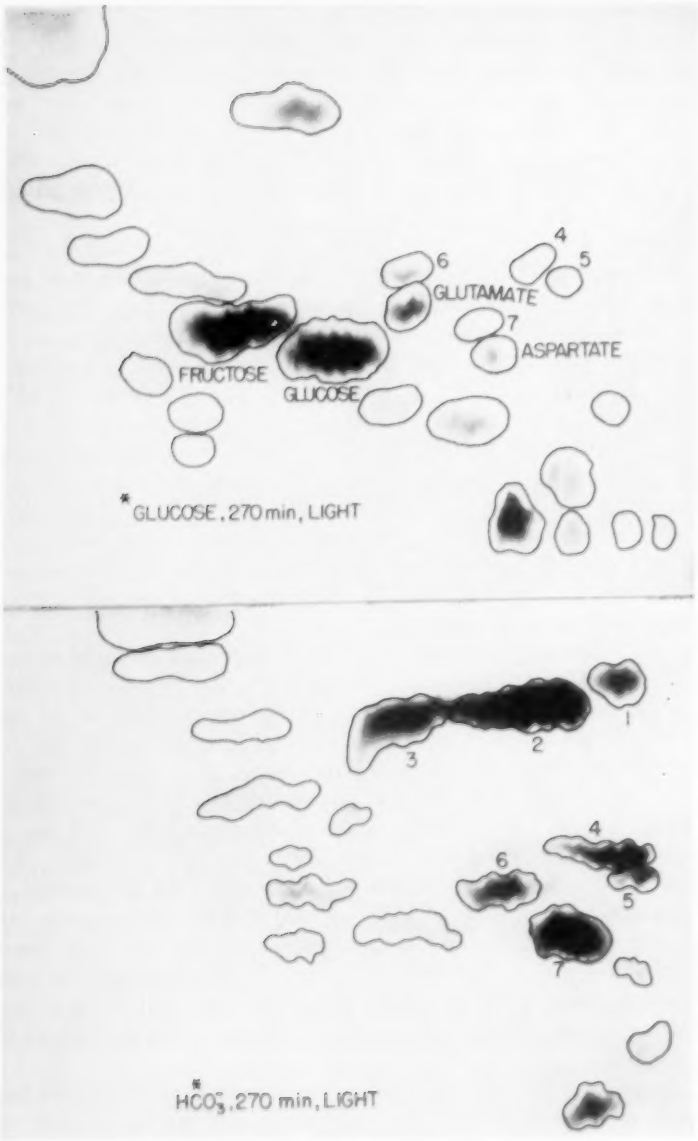
4
5
6
GLUTAMATE
7
ASPARTATE
FRUCTOSE
GLUCOSE
*GLUCOSE, 270 min, LIGHT
1
2
3
4
5
6
7
*HCO3, 270 min, LIGHT

FIG. 2.

in the dark; (3) after 60 minutes, both glutamate and aspartate, as well as lactate, succinate, malate, isocitrate, ketoglutarate, a compound tentatively identified as oxalate, and traces of glycolate were labeled; and, in particular, (4) after 270 minutes, illumination had induced a 100 to 300% increase in the amount of labeled succinate but a decrease in the amount of labeled ketoglutarate as compared to that in the dark.

Analysis of the bicarbonate-free series containing labeled glucose (*cf.* FIG. 2, top, for pattern) showed that: (1) as before, fructose was not utilized; but, (2) after 60 minutes, labeled glutamate appeared whereas no radioactivity was detectable in areas corresponding to ketoglutarate, isocitrate, malate, and succinate (however, labeled ketoglutarate, isocitrate, and malate were detectable after 270 minutes of incubation); and (3) even after 270 minutes, almost no labeled succinate was detected, the main, acidic, non-nitrogenous, labeled constituent being lactic acid. Thus, in the absence of bicarbonate, (a) the glutamate became labeled long before the intermediates of the tricarboxylic acid cycle, and (b) illumination did not induce the pronounced accumulation of labeled succinate that it did in the presence of unlabeled bicarbonate.

An examination of the series in which only the bicarbonate was labeled revealed (FIG. 1g) that after 270 minutes: (1) in the absence of glucose, about 70% more CO_2 was fixed in the light than in the dark; in the presence of unlabeled glucose, the increase was lowered to about 35%; and (2) both in the light and the dark, glucose depressed the rate at which CO_2 was fixed. In terms of actual fixation levels, about 3.2% and 1.8% of the labeled bicarbonate had been incorporated by *Blastocladiella* in the light and dark, respectively, when glucose was present.

Following resolution by chromatography, most of the labeled bicarbonate was recovered in fumaric, succinic, lactic, malic, isocitric, ketoglutaric, and presumably oxalic acid, with the rest of it distributed (FIGS. 1i and 2, bottom) among another dozen or so very lightly-labeled compounds (exception: the strongly-labeled unidentified compound in the sugar phosphate area; (*cf.* lower right hand corner in

FIG. 2. Top. Typical pattern for the distribution of labeled compounds recovered from illuminated non-proliferating suspensions of *B. emersonii* in uniformly labeled glucose and fructose and incubated for 270 minutes. Spots 4, 5, 6, and 7 correspond to malate, isocitrate, oxalate, and ketoglutarate, respectively. The uppermost, centrally-located spot above the glucose and fructose is lactate (*cf.* context for details). Bottom. Typical pattern for the distribution of labeled compounds recovered from illuminated, non-proliferating suspensions of *B. emersonii* in labeled bicarbonate and incubated for 270 minutes. Spots 1, 2, 3, 4, 5, 6, and 7 correspond to fumarate, succinate, lactate, malate, isocitrate, oxalate, and ketoglutarate, respectively (*cf.* context for details).

FIG. 2, bottom). When all radioactive areas were counted, recovery of C^{14} was: 100% and 93% for suspensions incubated in the light and dark, respectively, in the absence of glucose; and 92% and 96%, respectively, in the presence of glucose.

The significant findings were these: (1) there was no qualitative difference in the labeling pattern for illuminated and non-illuminated suspensions; (2) neither glutamate nor aspartate was labeled, although both were easily detectable in the pool of unlabeled amino acids in *Blastocladiella* and both had become labeled (FIG. 2, top) when glucose was the source of C^{14}; (3) there did occur a significant quantitative difference in the labeling pattern for illuminated and non-illuminated suspensions; *e.g.*, first, the sharp 75% increase in labeled succinate obtained in the absence of glucose, and the 26% increase obtained in the presence of glucose; and second, the decrease in labeled ketoglutarate.

IN VITRO STUDIES WITH CELL-FREE EXTRACTS OF B. EMERSONII. *A priori*, it was assumed that the increased growth and CO_2 fixation induced by illumination must have been due to one or more light-sensitive enzyme systems in *B. emersonii*. Subsequently, one such system was demonstrated *in vitro*.

Previous studies (**8**, **9**) with cell-free preparations of the thin-walled, colorless plants of *B. emersonii* established the presence therein of almost all of the enzymatic, oxidative activities usually associated with the tricarboxylic acid cycle. On the other hand, in the presence of cell-free extracts of resistant sporangia of *B. emersonii*, reduced TPN (generated *via* oxidation of polyphenolic substrates) appeared to drive a reductive carboxylation of ketoglutarate (**7**). A TPN-specific isocitric dehydrogenase was always present in these cell-free preparations, but other enzymes such as cytochrome oxidase and succinic dehydrogenase had apparently disappeared during the development and maturation of resistant sporangial plants.

In vitro studies with unlabeled compounds. For our *in vitro* investigations, therefore, we chose to study extracts of these resistant sporangial plants rather than thin-walled plants; it had seemed that investigations of carboxylation reactions involving ketoglutarate and isocitrate would be facilitated by the absence of the greatest possible number of other enzyme systems directly or indirectly coupled to isocitric dehydrogenase. Cell-free extracts of resistant sporangia were prepared according to Cantino and Horenstein (**7**), but they were employed at far lower concentrations than usual (see legend, FIG. 1h). Undialyzed preparations of this sort, in addition to their deficiency of cytochrome oxidase and succinic dehydrogenase, were also incapable of oxidizing

malate and cis-aconitate. Like the more concentrated preparations of Cantino and Horenstein, however, they oxidized isocitrate as measured by reduction of TPN at 340 mμ, but at much reduced rates because of the decreased concentrations of enzyme employed (FIG. 1h). When reaction mixtures were illuminated, a small but distinct inhibition occurred. In the absence of isocitrate, TPN was not reduced. In the presence of 10^{-2} M bicarbonate at the same pH, the initial rates of reduction of TPN were depressed by at least one-half and often almost to endogenous levels.

These same preparations also mediated the enzymatic oxidation of reduced TPN, as followed at 340 mμ (FIG. 1e). Addition of ketoglutarate and bicarbonate increased these rates and prevented them from tapering off as rapidly as those obtained in their absence. When reaction mixtures containing ketoglutarate were illuminated, a further increase in rates occurred (FIG. 1e). The magnitude of the stimulation was greatest at the beginning of the reaction. Relative rates of oxidation of reduced TPN in the light and dark at different stages of the reaction are delineated in FIG. 1d and illustrate how the stimulatory effect of light—highest at the start—gradually decreased as the oxidation continued. The stimulatory effect of light was detected in all instances where the enzyme concentration was low enough so that rates of oxidation of reduced TPN were of the order of magnitude shown in the figures. With enzyme concentrations appreciably greater than these, the reaction proceeded so rapidly that it was impossible to observe the effect of illumination.

Uni-dimensional chromatographic resolution of reaction mixtures in butanol-formic acid revealed that, as the reduced TPN was oxidized, (1) small amounts of succinate and isocitrate accumulated; (2) judging only from the intensity of the spots, no significant amounts of ketoglutarate seemed to disappear; and (3) a compound was produced which reacted with ninhydrin to yield a barely-perceptible yellow spot (rf just above that of glutamate in butanol-acetic acid; clearly not proline or hydroxyproline) which exhibited a very strong yellow-green fluorescence under both short-wave and long-wave U. V. light. The latter compound, like the succinate, accumulated only when ketoglutarate was present. The ketoglutarate used gave no such reaction with ninhydrin. The fluorescent substance was produced in both illuminated and non-illuminated reaction mixtures, but it accumulated much more rapidly in the light.

In vitro studies with labeled bicarbonate. Finally, experiments were initiated to provide definitive evidence that, in the foregoing study, CO_2

was indeed fixed, *in vitro*, by reductive carboxylation of ketoglutarate and, if possible, that the carboxylation was stimulated by light. Cell-free extracts were prepared as before, but from a different batch of dried, resistant-sporangial plants. Four different types of 7 ml reaction mixtures were prepared in duplicate: (1) labeled bicarbonate, 4.9 μM (specific activity, 18 μc per μM); unlabeled bicarbonate, 60.6 μM; α-ketoglutarate, 144 μM; reduced TPN, 500 μg; and supernatant from homogenate of resistant sporangia (700 mg dry weight per 35 ml M/15 phosphate, pH 7.0), 2.6 ml; (2) as in (1), but lacking reduced TPN; (3) as in (1), but lacking ketoglutarate; and (4) as in (1), but lacking both reduced TPN and ketoglutarate. All four types of reaction mixtures were incubated in the light and dark for 5, 20, 30, and 45 minutes. The reaction was stopped by addition of hot absolute alcohol to yield a final concentration of 80%. After acidification with acetic acid to release unfixed bicarbonate, solutions were concentrated in a vacuum desiccator to exactly 1 ml, centrifuged, and aliquots of the supernatant counted for total radioactivity, chromatographed, and radioautographed. The results (TABLE II) left no doubt that: (1) CO_2 was fixed *in vitro*, but only if both reduced TPN and ketoglutarate were present; in the absence of either one, practically no fixation occurred; (2) about 30% more CO_2 was fixed in the light than in the dark during the first 5 minutes of the reaction, the acceleration of CO_2 fixation by illumina-

TABLE II

FIXATION OF $C^{14}O_2$ BY CELL-FREE PREPARATIONS OF B. EMERSONII (CF. CONTEXT AND MATERIALS AND METHODS FOR FURTHER DETAILS)

Counts per minute (calculated as c/m per volume of supernatant equivalent to 1 mg dry weight of *B. emersonii*)		Components of reaction mixture (in all instances, the amount of supernatant per ml of reaction mixture was equivalent to 7.42 mg dry weight of *B. emersonii*)	Time of incubation (minutes)
Light	Dark		
2	2	$HC^{14}O_3^-$	45
0	3	$HC^{14}O_3^-$, TPNH	45
4	—	$HC^{14}O_3^-$, TPNH, pyruvate	45
0	0	$HC^{14}O_3^-$, pyruvate	45
6	—	$HC^{14}O_3^-$, DPNH, succinate	45
30	10	$HC^{14}O_3^-$, TPNH, succinate	45
6	0	$HC^{14}O_3^-$, ketoglutarate	45
70	54	$HC^{14}O_3^-$, TPNH, ketoglutarate	5
122	—	$HC^{14}O_3^-$, TPNH, ketoglutarate	20
124	115	$HC^{14}O_3^-$, TPNH, ketoglutarate	30
156	160	$HC^{14}O_3^-$, TPNH, ketoglutarate	45

tion decreasing to zero as the time of incubation increased; and (3) chromatographs of the reaction mixtures incubated for 45 minutes revealed that about 80 to 90% of the label was recovered in the compound presumed to be oxalate and the ketoglutarate itself. About 10% was recovered in isocitrate, and under 5% in succinate. The fluorescent compound, easily detectable once again, was not labeled.

Finally, to establish whether or not other carboxylations associated with the tricarboxylic acid cycle were mediated by these cell-free preparations, the latter were also incubated for 45 minutes in the light and dark with: (1) pyruvate, reduced TPN, and labeled bicarbonate; (2) pyruvate and labeled bicarbonate; and (3) succinate, reduced TPN (and, in replicate experiments, reduced DPN), and labeled bicarbonate. Apparently, the pyruvic acid was not carboxylated. But, succinate did induce CO_2 fixation at a level about one-fifth of that induced by ketoglutarate. Presumably, it was carboxylated to yield more ketoglutarate.

The foregoing experiments demonstrated *in vitro* that: (1) isocitrate was oxidized *via* a TPN-specific reaction and it was inhibited by bicarbonate and by light; (2) a mixture of bicarbonate and ketoglutarate stimulated the enzymatic oxidation of reduced TPN while part of the ketoglutarate was converted to a mixture of isocitrate, succinate, and presumably oxalate; both of these reactions were accelerated by light; (3) during carboxylation of unlabeled ketoglutarate with labeled CO_2, most of the label found its way into oxalate and back into ketoglutarate; and (4) in the presence of reduced TPN, succinate induced fixation of CO_2; we believe it was carboxylated to yield more ketoglutarate. Thus, it appeared as if illumination exerted a real influence upon the rate of turnover of, and the direction taken by, the tricarboxylic acid cycle in *Blastocladiella*. Thus, these observations not only provided some evidence for our postulations concerning the relation between carboxylation reactions and growth in *Blastocladiella*, but they were also consistent with and lent support to our concept of longer standing on the relation between carboxylation reactions and morphogenesis in *Blastocladiella* (**7**).

DISCUSSION

For the past five years, our work upon the relation between cellular metabolism and morphogenesis in *Blastocladiella emersonii* has centered, in the main, upon the notion (**4**, **5**, **6**, **7**, **9**) that: (1) the developmental pathway leading to the formation of resistant sporangial plants in *Blastocladiella* is triggered off by increased concentrations of bicarbonate; (2) the primary locus of this bicarbonate "trigger mechanism"

is the ketoglutarate oxidase system in a weakly-functional tricarboxylic acid cycle; (3) at the start of and during morphogenesis, bicarbonate helps to slow down, stop, and then reverse part of the tricarboxylic acid cycle; and (4) concomitant with this developmental pattern: (a) the terminal cytochrome oxidase is lost and replaced by a polyphenol oxidase; and (b) aconitase and the other enzymes of the Krebs cycle in the sequence leading from ketoglutarate to malate disappear or are rendered almost inoperative, while the isocitric dehydrogenase, needed for reversing the cycle by reductive carboxylation of ketoglutarate, remains fully functional.

Then, during the current investigations, we observed that both *B. emersonii* and a spontaneous mutant derived therefrom proliferated more rapidly in the light than in the dark. The mutant, in contrast to the wild type, apparently lacks ketoglutarate oxidase and aconitase; but, like the wild type, it does possess the other enzymatic oxidative activities usually associated with the tricarboxylic acid cycle (**8**, **9**). Illumination appeared to stimulate growth mainly during the first half of the generation time of populations grown in liquid culture. It seemed possible that the increased growth might have been due to increased rates of CO_2 fixation. Experiments with pre-formed, non-proliferating plant suspensions of *B. emersonii* then revealed that bicarbonate (as well as glucose) was, indeed, assimilated more rapidly in the light than in the dark. Consequently, we chose the working hypothesis that: (1) directly or indirectly, light was accelerating one or more carboxylation reactions; and (2) that the substrate at the presumed locus for the bicarbonate "trigger mechanism" in morphogenesis, α-ketoglutarate, might have been a starting point for one such carboxylation. If this were so, we expected that illumination might have lowered the ketoglutarate pool in the organism as the latter became reductively carboxylated to isocitrate. It followed, in turn, that little leakage of ketoglutarate would occur *via* other paths such as transamination to glutamate, and that some mechanism would be available for the rapid withdrawal of newly-formed isocitrate from the site of action. The latter might have involved conversion of isocitrate, perhaps *via* a citritase or *iso*citrase system (**15**), to succinate and a C_2 fragment such as glyoxalate, glycolate, or even some other C_2 compound such as oxalate derived therefrom.

The distribution of labeled compounds resulting from the fixation of $C^{14}O_2$ by pre-formed plants of *B. emersonii* was consistent with our hypothesis: (a) no detectable label appeared in the glutamate pool in the fungus; (b) illumination did induce a decrease in the amount of

labeled ketoglutarate; (c) a labeled compound, tentatively identified as oxalate, was recovered as a product of CO_2 fixation; and (d) illumination did bring about a sharp increase in the amount of labeled succinate. In fact, the % increase in labeled succinate was of the same order of magnitude as the % increase in CO_2 fixation induced by illumination.

The fact that unlabeled glucose depressed CO_2-fixation also lent support to our hypothesis. With glucose as a carbon source, *Blastocladiella* apparently carries on an almost-pure homolactic fermentation during growth (**6**); it follows that very little leakage occurs into the tricarboxylic acid cycle *via* pyruvate, thus implicating a weak C_2-forming mechanism. The results of the present study again showed that lactate was the principle, labeled, acidic, non-nitrogenous constituent resulting from metabolism of labeled glucose. Nevertheless, glucose would represent a potential force tending to maintain, even if only in small measure, the tricarboxylic acid cycle in its normal direction and, therefore, tending to decelerate any carboxylations which might occur *via* reversal of the cycle. At the same time, if the tricarboxylic acid cycle is only very weakly functional in its normal direction, *Blastocladiella* must derive most of its internal pool of glutamate and aspartate from alternate mechanisms not directly linked to the Krebs cycle *via* transamination. Compounds such as ketoglutarate could then arise by transamination from glutamate, rather than *vice versa*, and the ketoglutarate in turn could be further metabolized either *via* reductive carboxylation in the presence of CO_2 or oxidative decarboxylation in its absence.

With glucose as the sole source of C^{14} in our experiments with nonproliferating plants, the appearance of labeled glutamate did precede that of labeled ketoglutarate and other Krebs cycle intermediates. In the absence of bicarbonate, illumination had no effect on this pattern. But, when unlabeled bicarbonate was made available during metabolism of labeled glucose, tricarboxylic acid cycle intermediates appeared simultaneously with glutamate and aspartate. Under these conditions, light again induced large quantities of labeled succinate to accumulate while the small, labeled pool of ketoglutarate was depressed.

Finally, our experiments with cell-free preparations of *B. emersonii* provided more direct evidence for: (*1*) reductive carboxylation of ketoglutarate to isocitrate, and apparently the further conversion of isocitrate to succinate and oxalate; (*2*) a carboxylation dependent upon the presence of succinate and reduced TPN, suggesting the reductive carboxylation of succinate to yield ketoglutarate once again; and (*3*) the stimulatory effect of light upon the overall reaction. These phenomena were demonstrable *in vitro*. At the moment, we are in no position to ascer-

tain whether or not light has a direct stimulatory action on isocitric dehydrogenase, the enzymes (citritase?) which convert isocitrate to succinate and oxalate, or other reactions leading to the accumulation of "reducing power" which indirectly accelerates the carboxylation of ketoglutarate. Actually, the data in FIG. 1 tend to mitigate against a light-induced accumulation of "reducing power" in the form of reduced TPN. But, whatever the locus of the light effect, the fact remains that radiant energy seems to drive a reductive carboxylation of ketoglutarate to yield a net synthesis of succinate and a C_2 fragment tentatively identified as oxalate.

As a result of these experiments, we tentatively conclude (*cf.* FIG. 3) that the tricarboxylic acid cycle is not the main—and certainly not the only—source of supply for biosynthesis of the glutamate pool found in *Blastocladiella;* rather, its synthesis is dependent upon mechanisms other than the Krebs cycle. Thus, with pre-formed glutamate supplied from the external environment during growth, or with glucose as a source of glutamate, some of the intermediates of the tricarboxylic acid cycle may arise *via* the bottom door of the cycle. In the presence of CO_2, light energy helps drive the reductive carboxylation of ketoglutarate to isocitrate and thence, perhaps *via* citritase, to succinate and oxalate. At the same time, CO_2 not only prevents the further oxidation of succinate by means of the tricarboxylic acid cycle (**4**, **5**, **6**, **9**) but, on the contrary, is utilized for the reductive carboxylation of some of the succinate to yield more ketoglutarate. The excess succinate and the C_2 fragment (oxalate) thus formed must be metabolized further and integrated into the metabolic machinery of the plant; somehow, this yields the increased growth which results from the effect of illumination upon the S. K. I. (succinate-ketoglutarate-isocitrate) cycle.

Our studies of the stimulatory effect of illumination on growth of the mutant strain of *Blastocladiella* were approached independently and by different techniques which complement the investigations of the wild type. Without further elaboration, we think it is justified to say that the results derived therefrom are consistent with and offer some additional evidence for our explanation of the light effect on *Blastocladiella.*

The foregoing discussion has centered around our interpretation of experimental results derived from studies of the effects of light upon the metabolism of *Blastocladiella.* It is possible that further observations will necessitate some modification of this interpretation. But, these studies were motivated by our observations that light induced a distinct acceleration of growth and CO_2 fixation by *Blastocladiella.* We believe that these observations are intrinsically significant even though the underlying mechanism may not be fully understood at this time.

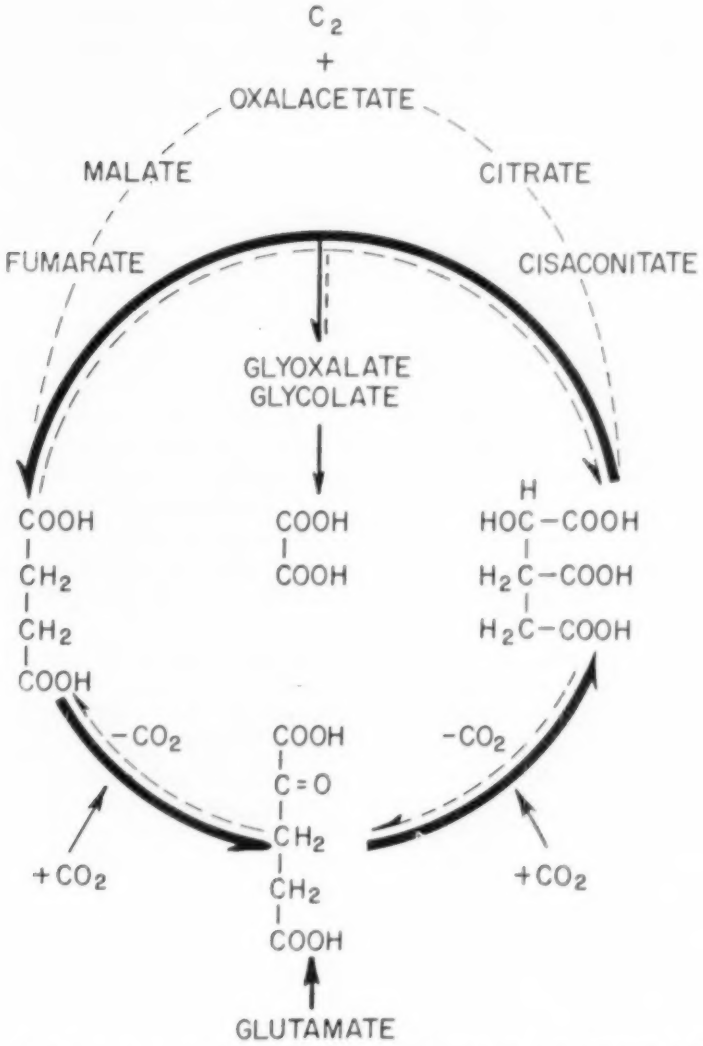

FIG. 3. The S. K. I. (succinate-ketoglutarate-isocitrate) cycle in *Blastocladiella*.

The aquatic Phycomycetes or water molds do not contain chlorophyll —indeed not even plastids—and they are usually considered to be the most primitive of the true fungi. *Blastocladiella*, on morphological as well as physiological (6) grounds, represents one of the most primitive

of these aquatic Phycomycetes. As a consequence, perhaps *Blastocladiella* should be relegated to a new and rather unique ecological niche in the economy of fresh-water lakes and streams, and by the same token to a new and unique niche in our conceptual framework of physiological evolution.

SUMMARY

The aquatic fungus *Blastocladiella emersonii*, and a carotenoid-bearing mutant derived therefrom, grew more rapidly in the light than in the dark. The accelerated growth of *B. emersonii* was attributed to a direct effect of illumination not mediated by carotenoid. Experiments with labeled and unlabeled bicarbonate revealed that illumination induced a large increase in CO_2 fixation over dark fixation levels, and concomitantly a large increase in the labeled succinate and a decrease in the labeled ketoglutarate pools in the organism. Similarly, both labeled and unlabeled glucose were consumed more rapidly in the light than in the dark.

Cell-free preparations of *B. emersonii* mediated an enzymatic, TPN-dependent oxidation of isocitrate which was inhibited by bicarbonate and light. These same preparations mediated an enzymatic oxidation of reduced TPN which was accelerated by ketoglutarate and bicarbonate; simultaneously, ketoglutarate was carboxylated. These reactions were further accelerated by light.

The mechanism of the effect of illumination was tentatively interpreted in terms of a light-stimulated, cyclic process, the S. K. I. cycle. This involved carboxylation of ketoglutarate, *via* isocitric dehydrogenase and perhaps citritase, to succinate and oxalate, and the further carboxylation of some of the succinate to yield ketoglutarate once again.

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IN VITRO EFFECT OF MYCOTHRICIN ON PLANT PATHOGENIC BACTERIA AND FUNGI

G. Rangaswami

The isolation and characterization of mycothricin, a new basic antibiotic belonging to the streptothricin group, was recently reported from this laboratory (**8**). The antibiotic is characterized by its activity against gram-positive and gram-negative bacteria, as well as against yeasts and filamentous fungi. In view of the recent interest in antibiotics possessing antibacterial and antifungal properties for the treatment of plant diseases, the activity of mycothricin against plant pathogens was investigated.

Streptomycin has so far been found most promising for controlling bacterial diseases (**3, 7, 12, 13**). A number of other antibiotics, notably actidione, antimycin, helixin B, antibiotic XG, and candicidin, have been found effective in checking fungal infections in small scale experiments (**1, 4, 5, 6, 11**).

EXPERIMENTAL RESULTS

Materials and methods. A partially purified preparation of mycothricin, with 400 units[1]/mg activity, was obtained from a strain of *Streptomyces lavendulae* (Waksman & Curtis) Waksman & Henrici, strain *3716* of the Institute of Microbiology Culture Collection. The agar-streak dilution method (**9, 10**) was first used for testing its effect on plant pathogenic bacteria and fungi. The test bacteria were grown on nutrient-glucose-agar for 18 hours. The cultures were now streaked on the same medium in Petri plates containing serial dilutions of the antibiotic, and incubated at 32° C for 24 hours. The fungi were tested in a similar manner using potato-dextrose-agar (PDA) medium; five-day sporulating cultures were suspended in sterile water, and the spore suspension streaked with a loop on the agar medium and incubated at 28° C for three days. The minimal concentration of the antibiotic required for complete inhibition of each organism was recorded (Table I).

[1] One unit is equivalent to the quantity of mycothricin/ml required for complete inhibition of the growth of an eighteen-hour culture of *Escherichia coli* on nutrient agar medium at 37° C.

TABLE I

INHIBITION OF PLANT PATHOGENIC BACTERIA AND FUNGI BY MYCOTHRICIN. AGAR-STREAK DILUTION METHOD USED

Test organism	Disease caused	Minimal inhibitory concentration: mcg/ml
Bacteria:		
Bacterium stewartii	Wilt of corn	25.0
Erwinia atroseptica	Black-leg of potato	0.5
Xanthomonas campestris	Black rot of crucifers	5.0
X. hederae	Leaf spot of ivy (*Hedera helix* L.)	10.0
X. phaseoli	Halo blight of beans	5.0
X. pruni	Leaf spot of plum, peach and apricot	1.0
X. vesicatoria	Bacterial spot of tomato and pepper	5.0
Fungi:		
Alternaria oleraceae	Leaf spot of brassicas	5.0
Aspergillus niger	Common mold and storage pest on corn seed, onion, etc.	10.0
Fusarium oxysporum f. *lycopersici*	Wilt of tomato	10.0
F. lini	Flax wilt	10.0
Helminthosporium avenae	Leaf blotch of oats	5.0
Sclerotinia fructicola	Brown rot of plum, peach and cherry	2.5

For the slow-growing and non-sporulating fungal cultures, the effect of mycothricin was tested by inoculating PDA plates containing different concentrations of the antibiotic with 4 mm culture discs, cut from the rapidly growing region of seven-day cultures of the test organism on the same medium. The discs were inoculated at the center of each plate and incubated at 28° C for 10 days and the diameter of growth of the fungi recorded (TABLE II).

The effect of mycothricin on fungal spores was tested by the slide-germination method of evaluating fungicides, recommended by the American Phytopathological Society (2). Spores from a seven-day

TABLE II

EFFECT OF MYCOTHRICIN ON GROWTH OF PLANT PATHOGENIC FUNGI ON PDA MEDIUM

Test organism	Diameter of growth in mm Concentration of mycothricin (mcg/ml)							LD_{50} mcg/ml
	0.0	2.5	5.0	10.0	25.0	50.0	100.0	
A. oleraceae	64	34	32	12	0	0	0	2.8
H. avenae	62	54	46	21	0	0	0	6.5
F. lini	60	55	50	30	23	0	0	10.4
F. oxysporum f. *lycopersici*	80	76	65	45	31	0	0	10.5
S. fructicola	65	49	42	33	24	22	0	10.2

culture of the test organism were suspended in sterile distilled water, washed by centrifugation, resuspended in 0.1 percent orange juice, and allowed to germinate in cavity slides in moist chambers at 25° C. The percent germination of the spores was recorded after 24, 48, and 72 hours by counting 200 spores under each treatment from six fields selected at random on two slides (TABLE III).

Inhibitory concentrations of mycothricin for fungi and bacteria. The minimal inhibitory concentration of mycothricin (TABLE I) for plant pathogenic bacteria varied from 0.5 to 25.0 mcg/ml. *Erwinia atroseptica,* the causal organism of black-leg of potatoes, was highly susceptible to the antibiotic, whereas the corn wilt bacterium, *Bacterium*

TABLE III

EFFECT OF MYCOTHRICIN ON GERMINATION OF FUNGAL SPORES

Test organism	Mycothricin (mcg/ml)	Germination of spores, per cent		
		24 hours	48 hours	72 hours
A. oleraceae	0.0	90.0	93.0	93.5
	0.5	84.0	88.5	86.5
	1.0	71.0	70.0	72.0
	2.5	53.5	62.5	64.0
	5.0	0.0	6.0	5.0
	10.0	0.0	0.0	0.0
	25.0	0.0	0.0	0.0
S. fructicola	0.0	87.0	95.0	93.5
	0.5	69.0	81.0	83.5
	1.0	6.0	11.5	11.0
	2.5	2.5	9.0	8.0
	5.0	0.0	0.0	0.0
	10.0	0.0	0.0	0.0
	25.0	0.0	0.0	0.0

stewartii, required the maximum concentration. The inhibiting concentrations of the fungi were expressed in terms of inhibition of spore germination and subsequent growth. Comparatively more antibiotic is required for complete inhibition of growth of the fungi, as determined by the second method (TABLE II). This is due to the fact that the inoculum consisted of a mycelial mat taken from the periphery of the fungal colony. The growth of the fungi in the presence of increasing concentrations of the antibiotic became thinner, with few hyaline submerged and radiating hyphae giving a larger diameter of growth. The LD_{50} value for each fungus culture was determined by plotting the radial growth against the concentration of the antibiotic on a semi-log paper and taking the diameter of growth of the fungus in check plates as 100 percent.

Inhibition of fungal spore germination by mycothricin. The spore germination studies, using *Alternaria oleraceae* and *Sclerotinia fructicola*, have shown that a much lower concentration of the antibiotic is required for inhibition (TABLE III). The LD_{50} values for these organisms were 3.1 and 0.7 mcg/ml, respectively. The spores of *S. fructicola* were lysed at 50 and 100 mcg/ml concentrations of the antibiotic after 48 hours, but not those of *A. oleraceae*. No malformation of the germtubes was observed in either case.

DISCUSSION

Results are reported of the antibacterial and antifungal property of mycothricin against plant pathogens. The concentrations of mycothricin required for complete inhibition of growth of fungi in agar medium is greater than the inhibitory concentrations for spore germination. Since in plant infections fungal spores play a greater role than the mycelium, it is suggested that the inhibitory concentrations for spore germination be taken as the standard. The sample of mycothricin used in these studies was relatively impure. It is hoped that further purification will lead to increased potency of the antibiotic. In a preliminary experiment on the toxicity of mycothricin to seed germination, it was found that treating wheat, tomato, and cucumber seeds with 2500, 1000, and 500 ppm of the antibiotic in water for 30 minutes did not impair the germination of the seeds, and there were no apparent symptoms of stunting of roots or shoots due to the antibiotic treatment.

SUMMARY

The effect of mycothricin on plant pathogenic bacteria and fungi was tested *in vitro*. The antibiotic was found to be effective in inhibiting the growth of various organisms in agar media. Spore germination studies showed that a much smaller concentration of the antibiotic is required for inhibition. The LD_{50} values of the antibiotic for radial growth and for spore germination of certain plant pathogenic fungi are reported.

The author is indebted to Dr. S. A. Waksman, Director of the Institute of Microbiology for suggesting this investigation. He also wishes to thank the staff members of the Plant Pathology Department of Rutgers University for supplying some of the cultures used in these studies.

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OBSERVATIONS ON GYMNOASCACEAE. III. DEVELOPMENTAL MORPHOLOGY OF GYMNOASCUS REESSII, A NEW SPECIES OF GYMNOASCUS AND EIDAMELLA DEFLEXA [1]

HAROLD H. KUEHN

(WITH 31 FIGURES)

The two previous papers in this series (Kuehn, 1955a, 1955b) presented the developmental morphology of four species of *Myxotrichum*: *M. uncinatum* (Eidam) Schroeter, *M. emmonsii* Kuehn, *M. conjugatum* Kuehn and *M. thaxteri* Kuehn. Each of these species possesses a type of gametangial morphology which is distinct from the others, and it was demonstrated that gametangial morphology is useful in the separation of species. The purpose of this paper is to report observations made on one species of *Eidamella* and on two species of *Gymnoascus*. The experimental procedures and the types of media utilized were similar to those described in detail in the first paper of this series.

GYMNOASCUS

Baranetzky (1872), working with *Gymnoascus reessii*, was the first investigator to examine the developmental morphology of a member of the family. Baranetzky noted that prior to ascocarp formation two morphologically similar swellings arose side by side on a single hypha, one on each side of a transverse wall. These grew into small branches which twisted spirally about one another, and later became club-shaped. Each was then cut off by a septum from its parent hypha. No evidence of fertilization was observed as would be indicated by breakdown of the cell walls between the gametangia. However, in abnormal cases, when only one gametangium was present, development of the ascocarp pro-

[1] The writer wishes to express his sincere appreciation to Dr. Leland Shanor, under whose supervision this study was pursued, for his interest and helpful suggestions and for reading the revised manuscript. Grateful acknowledgment is made to Dr. R. K. Benjamin and Dr. C. W. Emmons for supplying cultures of the fungi examined, and to Dr. D. P. Rogers for making available the herbarium specimens of Gymnoascaceae of the New York Botanical Garden.

ceeded no further. Baranetzky assumed, therefore, that fertilization probably was necessary for further development. He reported that when development was normal, the end of one organ, the antheridium, became enlarged and the swollen terminal segment was cut off by a septum. The other coiling initial, the ascogonium, put out from its free end a thin cylindrical projection, which also was cut off by a septum. The cylindrical projection encircled the antheridium once, then became segmented into almost isodiametric cells. Usually not more than two of these cells grew out to become ascogenous hyphae which rebranched repeatedly to form a dense cluster. As a result, a thick tuft of short branches arose, each of which became swollen at its apex to form an ascus. Thin vegetative hyphae grew out from the base of the antheridium. These, plus branches of the ordinary vegetative hyphae, formed a covering, or peridium, around the gametangial initials. Baranetzky reported that some of the peridial hyphae became cuticularized and branched at right angles, with the ultimate tips ending in many short, completely straight and spine-like projections. No accessory (asexual) spores of any kind were noted.

Eidam (1880) isolated *G. reessii* again, and studied its development. However, he found the developmental pattern to differ from that described by Baranetzky. According to Eidam, the copulating branches were dissimilar from the first. The initiation of the ascocarp, he reported, originated by formation of a side branch from one cell next to a septum. This branch elongated and spiralled in a tight coil 8 to 10 times around the next cell which then enlarged to appear club-shaped. Both cells became filled with dense protoplasm. Often, the spiralling branch, the ascogonium, arose from a hypha other than the branch about which it coiled. The antheridium, after elongation, divided into 2 or 3 cells, the terminal cell of which enlarged. Following this, the tightly coiled ascogonium became loosened to some extent. Septa formed to divide the ascogonium into several cells from some or all of which lateral branches were produced. Of these, only those from the lower 2 or 3 spirals elongated. The other branches, those from the higher spirals, intertwined and branched repeatedly, with the ultimate branch tips producing asci. Eidam stated that Baranetzky cited his own figures as being typical of weak mycelia, while Eidam maintained he himself worked with normal material. A perusal of Baranetzky's paper by the author, however, has revealed no such statement as Eidam claimed. On the contrary, Baranetzky explicitly stated that his cultures were normal and vigorous. Eidam found no imperfect stage associated with the cleistothecia.

Gymnoascus reessii was reinvestigated by Brefeld (**1891**), who asserted that both Baranetzky and Eidam were incorrect in assuming that the asci were derived from a sexual process. According to Brefeld, a hypha produced a branch which coiled and branched often to form groups of asci without a second branch taking part. Brefeld maintained, therefore, that a sexual process was not involved. Occasionally, coils involving two branches were found, but he regarded these as abnormal conditions. Baranetzky illustrated such solitary branches as Brefeld described, but, as already has been pointed out, regarded them as anomalous cases which developed no further.

Dale (**1903**) not only confirmed Baranetzky's work, but also extended our knowledge of the developmental morphology of *G. reessii*. According to her, mature asci were formed in two months. No accessory spore types were found. She reported that the gametangia were formed as Baranetzky had described. Her account of the development also included reference to nuclear phenomena. Two uninucleate branches arose from a single hypha, elongated, and twisted once or twice around one another. The apex of each enlarged and then was cut off by a septum, after which the multinucleate terminal cells came into contact and fused by dissolution of the wall between them. This was demonstrated through the study of sectioned material. She stated that nuclear fusion was not seen, but that it probably occurred at this time. Following conjugation, the antheridium became larger and more spherical so that the ascogonium appeared to be laterally applied to the antheridium. A prolongation originated from the tip of the ascogonium, coiled about the antheridium, and became septate. From most of the cells of this septate structure there arose short, thick branches (the ascogenous hyphae). These rebranched and eventually formed a dense cluster. Nuclei moved into the ascogenous hyphae, leaving the conjugating cells empty. Dale stated that nuclear division probably occurred in the ascogenous hyphae as they elongated, although this process was not observed. However, she followed the nuclear divisions which occurred at the apices of the ascogenous hyphae. One nucleus divided to give 2, then 4, then 8 nuclei. As this process occurred, the apical cell of the ascogenous hypha enlarged to form the ascus.

Conard (**1940b**) published a brief note on the development of *G. reessii*. However, no additional information was presented.

Gymnoascus setosus is the only other species of *Gymnoascus* whose development has been reported. In a very brief description Eidam (**1882**) stated that the formation of the coil was the same as he had described previously for *G. reessii*. In the formation of the peridium

he found there was only a single mycelial branch which encircled the gametangial complex. The cells of this branch became thickened and deep brown, sending out on all sides thorny, pointed, long, straight branches which, in their turn, produced verticels of short setae.

Dale (**1903**) attempted to study the morphology of *G. setosus*, utilizing a strain which was originally isolated from an old bee's nest by Massee. Although her isolate never produced ascocarps, she reported the production of hyaline conidia which were cut off laterally from certain hyphae at the places where septa occurred.

OBSERVATIONS AND DISCUSSION

GYMNOASCUS REESSII Baranetzky

A single complete description of *G. reessii* cannot be found in existing literature. The individual descriptions are either incomplete in certain respects or differ from one another in some details. Therefore, it is desirable at this time to give a more complete description, including the cultural characteristics, for each of the two strains which were investigated in the present study.

G. reessii #60: On Yp.Ss. agar (Emerson, **1941**), the colonies grow rapidly and consist of restricted growth which appears flat and bacteria-like at first, but then becomes green and finally yellow-green, with a wrinkled surface. The reverse of the colony is tinged green and is wrinkled, with grooves radiating from the center. Ascocarps appear in 20 days at the periphery of the colony as numerous white tufts which become light red-brown. The ascocarps are not conspicuous since they blend with the vegetative hyphae.

This species evinces no growth upon Czapek's agar, but on Sabouraud's agar the colonies have a flat, bacteria-like type of growth which is hyaline at first, but turns white. The colonies are slow-growing and restricted; however, the center of the colony is erumpent and is marked with ridges and grooves. The reverse of the colony is colorless. Ascocarps are as described for this strain on Yp.Ss. agar.

G. reessii #185: On Yp.Ss. agar the colonies are restricted, with little or no aerial mycelium. The colonies are hyaline and distinctly bacteria-like in appearance at first, but become white with long white columns of hyphae up to 6 mm high. These hyphal columns may occur in the center of the colony, but they are especially abundant at the periphery. According to Dale (**1903**), these erect strands of aerial hyphae develop on wet media, but not on dry substrata. However, in this strain of *G. reessii* the erect strands appeared under both condi-

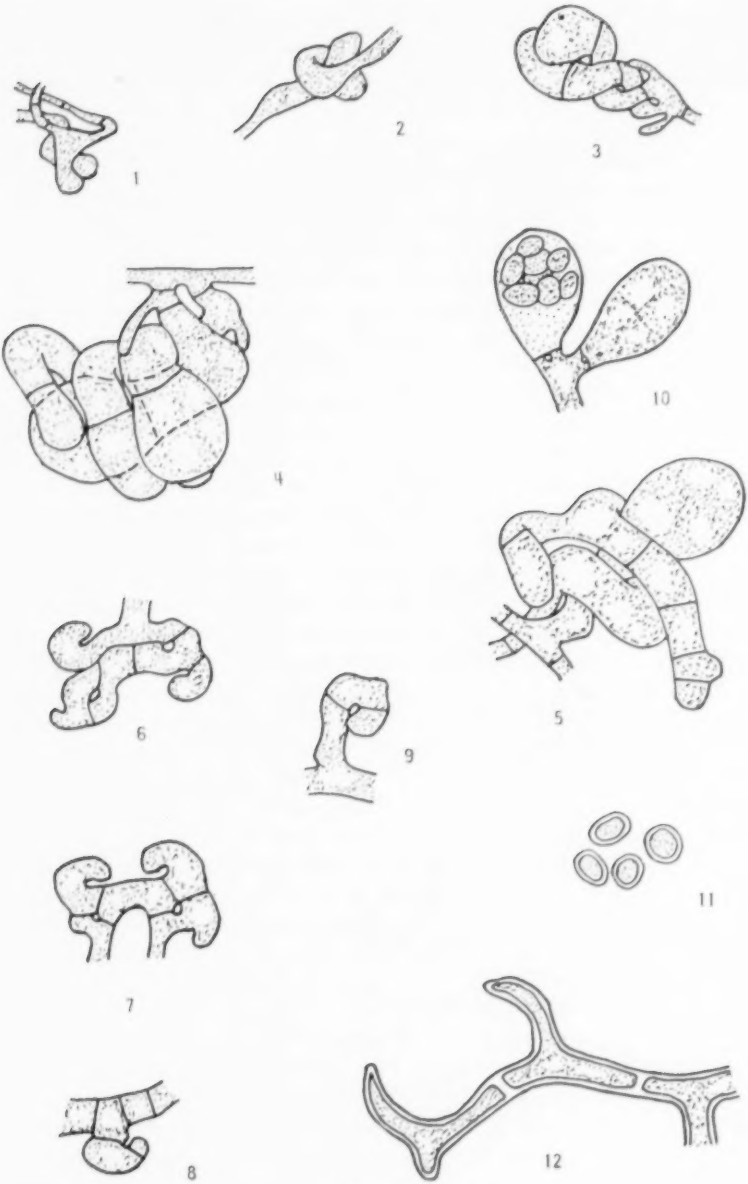
tions. Cleistothecia appear in 8–10 days as white tufts which turn yellow so that the surface of older portions of a colony is a brilliant yellow. As the ascocarps turn red-yellow and then red-orange the colonies assume the same shade. The reverse of the colony is tinged yellow.

Colonies on Sabouraud's maltose agar are restricted, hyaline, wrinkled and bacteria-like at first. Columns of hyaline hyphae rise up to 5 mm from the agar surface, but soon collapse to give a matted, woolly appearance to the colonies. Restricted, white mycelium appears in approximately 45 days, with the ascocarps developing in this mycelium as white tufts of hyphae and continuing their development as they do upon Yp.Ss. agar. The reverse of the colony is light orange-brown.

Cleistothecia, in both isolates, are spherical, light red-brown in strain *#60*, and brilliant red-orange in strain *#185*, measuring 189–441 μ diameter. Peridial hyphae are generally 2.8 μ in diameter, septate, cuticularized, in *#185* smooth and light yellow, turning red, but in *#60* asperulate and light red-brown. Peridial appendages are either simple, or divided terminally into usually two short, curved branches. Asci are hyaline, obovate, 8.4 × 11.4 μ, 8-spored, with the walls soon disintegrating and the spores remaining in a ball. Ascospores are smooth, light yellow, spherical, 2.8–4.2 μ diameter, or ovoid, 1.4–2.8 × 2.8–4.2 μ. Vegetative hyphae are hyaline, 1.2–1.6 μ in diameter, not including the racquet mycelium which is present. No imperfect spore phase is known.

Two strains of this species were investigated, both of which were isolated by R. K. Benjamin and bear his culture numbers. Strain *#60*, also designated as NRRL A-*3612*, was isolated from goat dung, September, 1948, Urbana, Illinois. Strain *#185* was isolated from rat dung, November, 1953, San Bernardino County, California.

These two strains are identical in their developmental morphology. The gametangia arise either side by side on the same hyphae (FIG. 2) or on different hyphae (FIG. 1). These morphologically similar short branches elongate and coil mutually about one another 2–3 times. Each initial becomes separated from its parent hypha by a septum. The distal end of each initial enlarges and is delimited as a large apical cell, while additional septa also are formed in each gametangium (FIG. 3). Fusion of initials by dissolution of the cell walls is not evident. From the enlarged terminal cell of one of the gametangia, designated as the ascogonium, a slender branch arises and proceeds to spiral about the apical cell of the antheridium (FIG. 4). The ascogonial prolongation becomes septate, producing ascogenous hyphae which recurve to form



FIGS. 1–12.

croziers (FIGS. 5, 8, 9). Ultimately, asci are formed from the penultimate cells of croziers (FIG. 10).

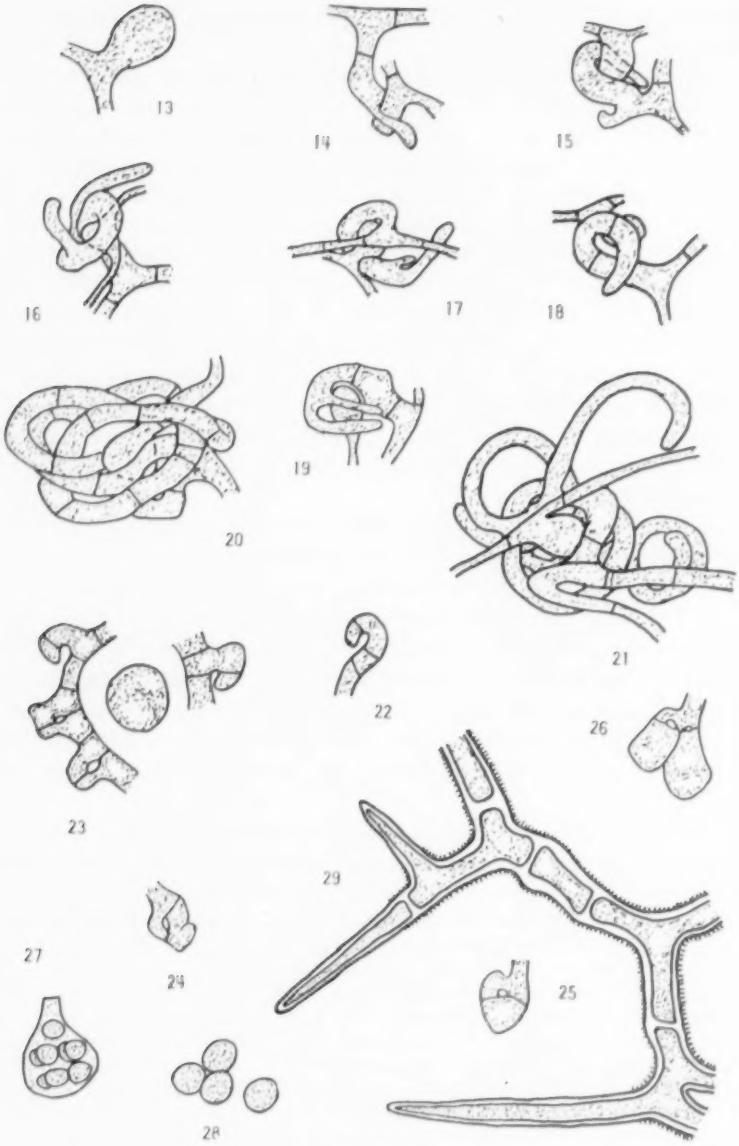
Prior to the formation of the ascogenous hyphae, slender vegetative hyphae arise from the lower portion of the coil and grow up around the initials. They do not take part in formation of the peridium, and later they can be detected from among the asci as delicate hyphae. The cleistothecium may be simple, but it is usually compound in that asci from several pairs of initials are enclosed within one peridium. The latter is formed from vegetative hyphae in the vicinity of the gametangia.

Nothing resembling what Eidam (**1880**) and Brefeld (**1891**) described for *G. reessii* in the initiation of the ascocarp ever has been observed during this study. Eidam claimed that an ascogonium coiled either about its parent hypha or about a neighboring hypha, and then produced ascogenous hyphae. Brefeld maintained that a single ascogonium became coiled and then produced ascogenous hyphae without the participation of an antheridium, a cell from the parent hypha, or from another hypha.

The reports of Baranetzky (**1872**) and Dale (**1903**) are in close agreement with the findings of the present investigation. All three investigations concur on the structure and development of the gametangial initials. However, Dale and Baranetzky found that in every instance the gametangia arose from the same parent hypha, while the author frequently has encountered initials which were produced upon separate hyphae. Benjamin (**1956**) illustrated croziers but did not discuss their development. The other investigators stated that the ascogenous hyphae branched often, and finally produced asci at the apices.

Conard (**1940a**) described a conidial stage for *G. reessii*. However, in the present study no conidial stage was found for this species. Since

FIGS. 1–12. *Gymnoascus reessii*. 1, 2. Young gametangia arising from separate hyphae. 3. Coiling initials arising from the same hypha. Each delimits an enlarged terminal cell. Vegetative branches grow from the base of the coil. 4. A prolongation grows from the terminal cell of the ascogonium and proceeds to coil about the central, septate antheridium. The prolongation is septate at this stage. 5. A septate prolongation with an ascogenous hypha originating as a small bulge. 6, 7. Portions of some septate prolongations with croziers. In several of the croziers the penultimate cell grows out to form additional croziers. 8, 9. Stages in crozier formation showing variation in the length of ascogenous hyphae before they form croziers. 10. Mature and immature asci. 11. Mature ascospores. 12. A portion of a peridial hypha showing the terminal, short, curved appendages. All figures × 1300.



FIGS. 13–29.

the existing literature is very vague and indefinite on the limits of species, identification of Gymnoascaceae is difficult unless a collection of various species is available for examination. Therefore, it seems likely that Conard may have been working with a species other than *G. reessii*.

Gymnoascus brevisetosus sp. nov.[2]

Cleistotheciis globosis, brunneis, 315–555 μ diam. Peridii hyphis septatis, asperulatis, 4.5–5.8 μ diam. Appendiculis e spinis, levibus vel asperulatis, ad basim 2.8–4.2 μ diam., 11.9–37.8 μ longis, constitutis. Ascis hyalinis, obovatis, 5.6–7.0 $\times$ 7.0–8.4 μ, octosporis. Ascosporis hyalinis, levibus, globosis, 2.7–3.0 μ diam. Hyphis sterilibus hyalinis, 1.2–1.6 μ diam. Oidiis hyalinis, 1.2–1.6 $\times$ 2.8–8.2 μ.

Cleistothecia spherical, brown, 315–555 μ diam. Peridium composed of septate, cuticularized, asperulate hyphae, 4.5–5.8 μ diam., with appendages of sharp spines, smooth or asperulate, 2.8–4.2 μ diameter at the base, and 11.9–37.8 μ long. Asci obovate, hyaline, 5.6–7.0 $\times$ 7.0–8.4 μ, 8-spored. Ascus wall ephemeral, with the spores remaining in a ball. Ascospores hyaline, smooth, spherical, 2.7–3.0 μ diam. Vegetative hyphae hyaline, 1.2–1.6 μ diam. Racquet mycelium also present. The imperfect phase consists of hyaline oidia, 1.2–1.6 $\times$ 2.8–8.2 μ.

Colonies on Sabouraud's maltose agar are white, grow rapidly, turn yellow in the center in about 18 days, and then become yellow-brown. Erect, hyaline tufts of hyphae up to $\frac{1}{2}$ cm high arise in the center of the colony, but these soon collapse. The reverse of the colony is tinged

[2] The writer acknowledges with appreciation the assistance of Dr. D. P. Rogers, New York Botanical Garden, for the preparation of the Latin diagnosis.

FIGS. 13–29. *Gymnoascus brevisetosus*. 13. Young antheridium arising as a short lateral branch. 14. Gametangia arising from separate parent hyphae. The ascogonium has a slender apical portion. 15, 16. The slender portion of the ascogonium coiling about the antheridium. A branch arises near the base of the ascogonium. 17. An ascogonium branching near the apex. 18. An ascogonium which is twice-septate. 19. A septate ascogonium with a slender apical region, and with a slender branch near the apex. 20, 21. The ascogonium, and the branches derived from it, coiling about the antheridium and becoming septate. 22. A crozier formed at the tip of an ascogenous hypha. 23. Portion of a coil as seen from the top. Septate ascogonium, showing short ascogenous hyphae with croziers in various stages of development. A central antheridium is present. 24. Penultimate cell of a crozier beginning to grow out to form an ascogenous hypha. 25. The penultimate cell of a crozier beginning to enlarge to become an ascus. 26. Stages in ascus formation at the apex of an ascogenous hypha. 27. A mature ascus. 28. Mature ascospores. 29. Portion of a peridial hypha showing spine-like appendages. All figures $\times$ 1300.

yellow. Cleistothecia appear in 6 weeks as white tufts of hyphae, and later become brown. Very few ascocarps are produced.

On PDA the colonies are white, sparse, and grow rapidly. Reverse of the colony is colorless, with a yellow tinge at the margins, but with age it becomes pink to red-orange. Cleistothecia, which are recognizable as dense white tufts in the sparse mycelium, may appear as early as 11 days after inoculation, or may not appear for 1½ months. The ascocarps become yellow-brown to brown as they mature.

Gymnoascus brevisetosus was isolated by Emmons (**1954**) from the lung of a prairie dog (*Cynomys ludovicianus*) in Texas. In his report the fungus was considered to be *G. reessii*, although cultures obtained from him were marked *G. reessii* ? *#5001*.

Gymnoascus brevisetosus is distinguished from all other species of *Gymnoascus* by its very small, spherical, smooth ascospores which measure 2.7–3.0 μ diameter. In addition, this species emits an extremely pronounced, musty, earthy odor. Also, oidia have not been reported for any other species of the genus. It is most easily recognized by the short unbranched spine-like appendages which terminate in sharp points and, because of this characteristic, the specific epithet *brevisetosus* was selected.

The ascocarp is initiated by gametangia which arise upon separate parent hyphae. The antheridium is a short, rather club-like, lateral branch (FIG. 13). The ascogonium is produced as a lateral branch of an adjacent hypha. A slender prolongation grows forth from the apex of the ascogonium and spirals about the antheridium (FIGS. 14, 15). During this time the ascogonium becomes delimited from its parent hypha by a septum. One or more branches arise from the ascogonium (FIGS. 15, 16, 17, 19) and grow about the antheridium to form a loose weft of hyphae (FIGS. 20, 21). Evidence which indicated the occurrence of gametangial fusion was not observed. Septa are formed in the ascogonium and its branches (FIG. 20), following which most of the resultant cells produce croziers (FIG. 23). The croziers are unique in that the ultimate cell fuses with the ascogonial cell adjacent to the cell which produced the crozier (FIG. 23). The initial croziers do not produce asci, but grow out to form additional croziers (FIG. 24). This process is repeated so that extensive groups of ascogenous hyphae form before the penultimate cells of croziers swell to develop into asci (FIG. 26).

Vegetative hyphae surroundings one or more groups of fertile hyphae differentiate and become thickened, cuticularized, yellow and asperulate.

The cleistothecia are compound, since within each are found asci derived from several pairs of gametangial initials.

EIDAMELLA

Eidamella spinosa, a dermatophyte isolated from dog ringworm lesions, was described by Matruchot and Dassonville (**1901**). This was the first dermatophyte known to produce cleistothecia. In the initiation of the ascocarp, these authors stated that either one initial arose and spiralled about its parent hypha, or more often, a short lateral branch coiled around an adjacent short, lateral branch. The spiral ascogonium became septate and each cell branched to give rise to an ascogenous hypha, from the terminal cell of which the ascus developed.

The wall which was formed was even more loosely organized than in *Gymnoascus*. The peridial hyphae were strongly cuticularized and ended in black spines whose extremities were hyaline and not cuticularized. Certain peridial hyphae possessed one to several twisted, terminal, hyaline spirals plus 2 to 3 lateral, hyaline spirals. According to Matruchot and Dassonville, these characters of ornamentation distinguished this new genus from related genera. Also, the asci were stalked whereas asci in the other Gymnoascaceae are sessile or nearly so. No imperfect stage was found.

Dodge (**1935**) placed *E. spinosa* in synonomy with *G. setosus*. DeLamater (**1937**), however, maintained that they were distinct. He reisolated *E. spinosa* from the fingernail of a patient in a Boston Hospital, studied its development, and, in general, agreed with Matruchot and Dassonville on the developmental pattern as they had reported it. The gametangia appeared as irregular coils, with or without knob-like central cells. No fusion was seen, but DeLamater stated that probably two adjacent cells of the coil itself fused, the daughter nuclei from these cells becoming associated as a dicaryon in ascogenous hyphae. Typical croziers were formed.

The description given by Matruchot and Dassonville of the development of the ascocarp in *Eidamella* was not extensive, and they overlooked the formation of croziers. DeLamater gave a more extensive report, described crozier formation, and provided two plates illustrating the developmental characteristics of this species. Therefore, there is no need for further illustrations. However, although the present study has confirmed the work of DeLamater, the developmental morphology has been elaborated with respect to several details and for this reason it is desirable to give a complete description of the species, including the cultural characteristics.

OBSERVATIONS AND DISCUSSION

EIDAMELLA DEFLEXA (Berk.) Benjamin, El Aliso **3**: 313. 1956.

Myxotrichum deflexum Berk., Ann. Mag. Nat. Hist. I. **1**: no. 122. 1838.

Eidamella spinosa Matr. & Dass., Bull. Soc. Myc. Fr. **17**: 123. 1901.

Eidamella papyricola Saccas, Bull. Soc. Myc. Fr. **66**: 121. 1950.

Colonies on Sabouraud's agar white, becoming grey as the dark cleistothecia develop. Reverse of the colony becoming red, with the

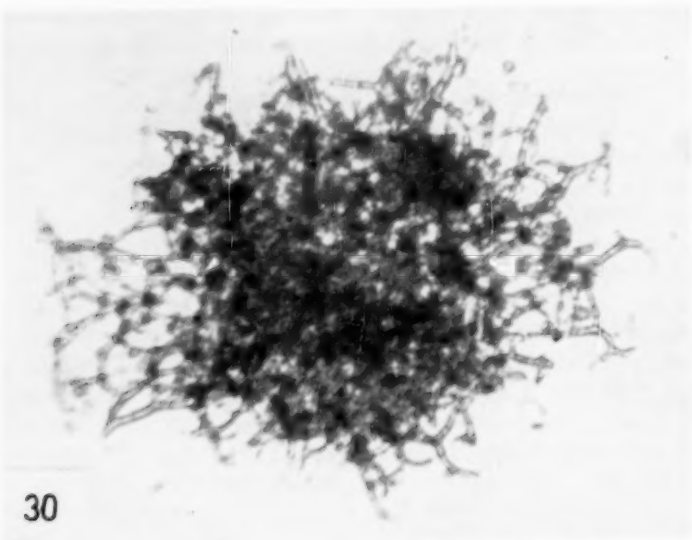


FIG. 30. Cleistothecium of *Gymnoascus reessii*, × 273.

pigment diffusing into the agar. Red as well as dark, almost black, droplets of exudate are formed on the surface of the colony. Cleistothecia appear in 2–3 weeks as black tufts of hyphae nestled in the mycelium.

Colonies on PDA are white; however, the hyphae which are in contact with the substratum turn slightly red. The aerial mycelium either remains white or becomes pink. Reverse of the colony is red, with the pigment diffusing into the substratum. The colonies turn grey with the advent of ascocarp production.

Colonies on pablum agar are slow-growing, restricted, thin, white, and remain so with age. The reverse of the colony is tinged red-orange, with the typical red pigment of this species, as produced on Sabouraud's and the majority of other media, absent. No pigment diffuses into the medium. Cleistothecia are usually absent, but they may be produced.

Cleistothecia are spherical, black, 126–352 μ diam., including the spines. Ascocarps frequently are much reduced, with 3 or 4 appendages surrounding a few asci. Peridial hyphae appear dark brown under the

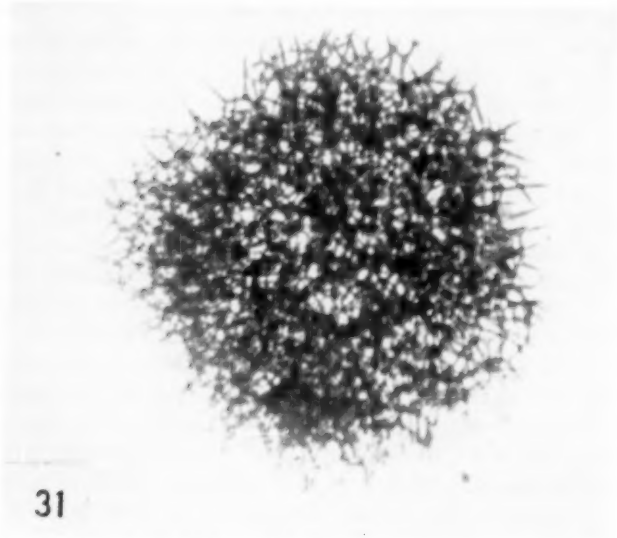


FIG. 31. Cleistothecium of *Gymnoascus brevisetosus*, × 137.

microscope, and are composed of septate, branched, cuticularized, smooth hyphae. The main trunk of an appendage is variable in length, up to 189 μ long, 1.4–2.8 μ diam. From the main trunk arise short, lateral branches which may be straight, but are usually deflexed, either upward or downward, 1.4–2.0 μ in diam., up to 56 μ long, ending in hyaline tips. Shorter appendages, found toward the interior among the asci, end in several hyaline spirals. The asci are hyaline, obovate, clavate, or ovoid, and measure 4.2–7.0 × 12.6–25.2 μ, including the long stalks. The ascus wall is ephemeral, with the eight spores separating when the ascus wall disintegrates. Ascospores are hyaline, ovoid to

elliptical, 2.4–2.8 × 3.6–5.2 μ. Vegetative hyphae are hyaline or red, 1.1–4.2 μ diam., not including the racquet mycelium which is present. No imperfect spore phase is known.

Several isolates of this species were studied. One isolate was present in the mycological culture collection at the University of Illinois. Originally this strain was obtained from the Centraalbureau voor Schimmelculture, Baarn, Holland. It is a subculture of DeLamater's isolate. Another strain which was examined was isolated by R. K. Benjamin, in March, 1952, from an old board in a basement in Belmont, Massachusetts. It was provided by Benjamin labelled *Myxotrichum deflexum* #57.

Initiation of the ascocarp may take place by one of 3 methods. The ascogonium may arise as a branch directly on one side of a septum. Then it proceeds to coil 4–5 times in a helix around the cell immediately next to it in the same hypha. This hyphal cell often becomes somewhat club-shaped. Another pattern of initiation involves an antheridium which arises as a short or elongated club-like branch from a hypha that also produces the ascogonial branch close to the antheridium. The ascogonium spirals around the antheridium in a tight or loose coil which may be quite irregular. The ascogonium may also arise as a coiling branch which has no associated central cell, whether it is an antheridium or a hyphal cell. No indication of gametangial fusion could be observed in this species. In any case, septation of the ascogonium occurs with some of the resulting cells giving rise to ascogenous hyphae. They remain short and soon form croziers which usually do not develop asci but instead grow out to form additional croziers. Fusion of ultimate and antepenultimate cells results in a cell from which an ascogenous hypha grows forth to produce a crozier. In this manner, several croziers arise from each ascogenous hypha and produce asci by enlargement of the penultimate cells.

The peridial hyphae arise principally as branches of the parent hypha below the coil, although surrounding vegetative hyphae often take part. Peridial branches are distinctive from the start in their greater diameter and characteristic right angle branching. Soon they darken and become almost black, with thick, cuticularized walls. The apices of the peridial hyphae and their lateral branches end in hyaline tips. Certain interior peridial hyphae terminate in 3–7 hyaline spirals. The peridium is very loose so that the asci are exposed and can be seen through the peridium.

Examination of the herbarium specimens of Gymnoascaceae from the New York Botanical Garden, and the exsiccati of Gymnoascaceae

present in the Mycological Herbarium of the University of Illinois, has shown that *Myxotrichum deflexum* Berk. is identical with *Eidamella spinosa* Matr. and Dass. In addition, several strains of *M. deflexum* were obtained from Dr. R. K. Benjamin, who had isolated them from widely scattered localities. These strains proved to be identical in external appearance and in cultural and morphological characteristics with DeLamater's isolate of *E. spinosa*. Berkeley's illustration of *M. deflexum* (**1838**), although it is only a sketch, definitely shows that this is the same species as *E. spinosa*. The peridium of this species does not correspond at all with that of the species of *Myxotrichum* and the author feels that Matruchot and Dassonville were justified in erecting the new genus *Eidamella* for this species. These studies by the writer were carried out in 1954. Recently, Benjamin (**1956**) reviewed the Gymnoascaceae in the most extensive study of this family to date. Benjamin came to the same conclusion as did the writer, and proposed the new combination *Eidamella deflexa*.

Recently, Saccas (**1950**) isolated, from books in a library, a new species, *Eidamella papyricola*, which in my opinion is only a strain of *E. deflexa*. His description of the development is not at all clear, and his illustrations are poor. However, the resemblance to *E. deflxa* is apparent. No croziers were described and the asci were said to be formed by "budding" of the ascogenous hyphae. Saccas stated that this new species differed from *E. spinosa* in two respects. First, the red pigment, which is typical of *E. spinosa*, is absent, and second, *E. papyricola* was isolated from paper, a habitat quite different from that of *E. spinosa*, which at that time had only been isolated from fingernails and from skin lesions of a dog. However, the present study has shown that *E. spinosa* does not always form this red pigment, depending upon the type of culture medium utilized, and the strains which were received from Benjamin as *M. deflexum* were found in habitats similar to that of *E. papyricola*. Therefore, the distinction made by Saccas between *E. spinosa* and *E. papyricola* proves to be inadequate for specific separation.

SUMMARY

1. The developmental morphology and cultural characteristics of *Gymnoascus reessii*, *G. brevisetosus*, a new species, and *Eidamella deflexa*, are discussed.

2. Diagnostic characteristics are presented for these three species.

3. The morphological development of *G. reessii* was found to be similar to what Baranetzky and Dale described, but quite different from what Eidam and Brefeld reported.

4. *Myxotrichum deflexum* Berk., *Eidamella spinosa* Matr. & Dass., and *Eidamella papyricola* Saccas represent the same organism.

5. The developmental morphology of *E. deflexa* was found to be essentially identical with that which DeLamater had previously reported.

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A PRELIMINARY SURVEY OF THE GENUS PLATYGLOEA

R. J. Bandoni

(with 9 figures)

The genus *Platygloea* was established by Schroeter (**1887**) to include three species of fungi with *Auricularia*-like basidia, but differing from that genus in form and texture. The first of these, *P. nigricans* Schroet. (*P. disciformis* (Fries) Neuhoff), occurs on dead twigs of linden and was believed by Schroeter to be identical with *Agyrium nigricans* var. *minus* Fries (**1822**). The following year (**1888**), the same fungus was fully described and illustrated by Brefeld as *Tachaphantium tiliae*. Höhnel (**1904**) and Neuhoff (**1924**) gave reasons for believing that *Achroomyces tiliae* Bonorden (**1851**) was an earlier name for the same species. Neuhoff reverted to the name *Platygloea* in a later paper (**1936**). It is possible that the species described and illustrated by Bonorden was identical, but it cannot be regarded as established. The descriptions by Schroeter permit accurate recognition of the species upon which he based the genus and there is enough doubt about *Achroomyces* to make it seem unwise to adopt that name at the present time.

Coker (**1920**) quotes Patouillard to the effect that *Helicogloea* should be merged with *Platygloea*, but the curious hypobasidial sac present in the former genus would seem to justify retention of it as distinct. The basidial characteristics and arid or sub-arid texture of *Helicobasidium* are approximated in several species of *Platygloea* and the two genera, as defined at present, are not clearly separated. Although the easily detached basidia of *P. pustulata* are suggestive of those which occur in *Mycogloea* and *Xenogloea*, these fungi appear to be unlike in other characteristics.

The genus now consists of a heterogeneous assortment of species, and it is possible that it may be divided into several more consistent genera when better known. Olive (**1951**) suggests that the group might eventually be broken down into two series, or subgenera, on the basis of the presence or absence of persistent "probasidia." In addition to the types of fructification listed by Olive in his redefinition of the genus, there occur pezizoid, discoid (both superficial and erumpent),

and erect, foliose forms. Most of the species are saprobic, but three are parasitic on other fungi. Basidiocarp form and parasitism have been used as basic characters in the delimiting of genera in other groups of Heterobasidiomycetes. It is clear that our knowledge of this group is, as yet, too fragmentary to justify an attempt at monographic treatment, but a preliminary survey may prove helpful.

Basidial characteristics have been used extensively in the following key and descriptions. Since there are, at present, several systems of basidial terminology in use, definition of the terms, as used here, is in order. The probasidium is the immature basidium, i.e., the elongate cylindrical to clavate structure (with or without spherical to clavate basal swellings or dilations) within which karyogamy and meiosis occur, prior to the development of protuberances. This is designated the basidium following the initiation of development of sterigmata, or of tubular extensions terminating in sterigmata, the epibasidia. If the basidium is divided into two distinct portions, an elongate cylindrical sporogenous part borne upon a spherical or clavate basal portion, then the term hypobasidium is used to designate the latter. The epibasidia are extremely variable in length and are not, therefore, included in the basidial lengths given.

PLATYGLOEA Schroet. Krypt-Fl. Schles. **3**[1]: 384. 1887.
?Achroomyces Bonorden, Handb. allg. Myk. 135. 1851.
Tachaphantium Brefeld, Unters. **7**: 78. 1888.
Tjibodasia Holterm. Myc. Unter. Trop. **44**. 1898.

Fructifications fleshy, gelatinous, or waxy, drying horny or arid, typically resupinate and expanded or pustulate, sometimes pezizoid and centrally attached, lacking in some parasitic forms, erect and foliose in one species, discoid and erumpent in the type; hymenium unilateral to amphigenous, smooth or tuberculate, with or without sterile elements; probasidia clavate to cylindrical or with a dilated basal portion (hypobasidium) and a more or less cylindrical sporogenous part, aseptate or becoming 1–4-septate; basidia straight or curved, the sporogenous cells developing epibasidia terminating in sterigmata and basidiospores, or the fertile portion protruding above the surface of the fructification and sterigmata then arising directly from the cells; basidiospores hyaline, white in mass (pinkish-yellow in *P. caroliniana*), globose to ovate, cylindrical, or allantoid, germinating by repetition, budding, or by germ tubes; hymenial conidiophores and conidia present in some species.

TYPE: *Platygloea nigricans* Schroeter.

KEY TO THE SPECIES

a. Growing on or in other fungus fructifications, presumably as parasites.....b
a. Saprobic, on wood, bark, litter, or dung, at most encrusting old fructifications.....d
b. On sphaeriaceous fungi; pale opalescent, pulvinate; basidia 120–300 μ in length.....1. *P. miedzyrzecensis*
b. On or in other basidiomycete fructifications; basidiocarp, if present, pallid to yellowish, effused; basidia mostly less than 60 μ in length.....c
c. Distinct fructifications lacking, growing within the basidiocarps of dacrymycetaceous fungi; hyphae without clamp-connections; hymenial conidia subglobose to ovoid, 3–5.3 × 2.3–3.7 μ.....2. *P. arrhytidiae*
c. Fructifications waxy to subgelatinous, effused, pallid to yellowish, lacking on gelatinous hosts; clamp-connections abundant; hymenial conidia long, ovoid to ellipsoid, 5–10(–12) × 3–4.5 μ.....3. *P. peniophorae*
d. Fructifications erect, foliose-lobate; hymenium amphigenous (Java).....4. *P. javanica*
d. Fructifications resupinate; hymenium more or less unilateral.....e
e. Pezizoid or discoid, sometimes anastomosing, attached by a point.....f
e. Pustulate, discoid or effused, broadly attached.....h
f. White; waxy; margins appressed to the surface; hymenium smooth; paraphyses long-clavate, thick-walled; basidiospores 7–9 × 4–6 μ.....5. *P. brunnea*
f. Yellowish; gelatinous or coriaceous; margins sinuous; hymenium venose; paraphyses, if present, slender.....g
g. Rosaceous yellow; gelatinous; paraphyses long, slender; basidia 90 × 5 μ; spores 10 × 5 μ.....6. *P. carnea*
g. Golden yellow; soft-coriaceous; paraphyses lacking (?); basidia 40–50 × 6 μ; spores 10–12 × 8 μ.....7. *P. succinea*
h. On dung of various herbivores; fructifications discoid, applanate, 1–4(–10) mm in diameter; pale flesh color to pale violaceous.....8. *P. fimetaria*
h. Not coprophilous.....i
i. Discoid, applanate, margins determinate.....j
i. Pustulate or effused.....k
j. Erumpent from the cortex of birch and linden as subrotund, flattened disks, 1.5–2 mm in diameter; basidia 90–180(–300) × 9–12 μ; spores 26–30(–36) × (6–)7.5–9 μ.....9. *P. disciformis*
j. Superficial, orbicular to elliptic, free at the extremities, 2–4 cm in diameter, surrounded by white or pale yellow strands of hyphae; on wood of *Lansium domesticum* (Java).....10. *P. fibrosa*
k. Pustulate, sometimes becoming confluent, but retaining pustulate character.....l
k. Effused from an early stage.....q
l. Basidia mostly non-septate, bearing a single terminal epibasidium and spore; spores 12.8–18.3 × 9.6–13.7 μ, becoming septate; on *Juniperus* wood.....11. *P. unispora*
l. Basidia 0–4-septate, each cell usually giving rise to an epibasidium and spore; spores remaining aseptate.....m

m. With emergent paraphyses extending 55–100 μ or more above the surface; basidia 0–4-septate, (38–)52–78(–135) × 5.5–9.6 μ; spores cylindrical to allantoid, (12.5–)16.5–25.5(–31) × (5.5–)6–7.5(–8) μ.....12. *P. longibasidia*

m. Paraphyses, if present, not emergent.....n

n. Basidia thread-like, up to 200 × 4 μ, mostly 3-septate; spores oval, 12 × 6 μ.....13. *P. blastomyces*

n. Basidia under 60 μ in length, rarely with more than two sporogenous cells.....o

o. Basidia commonly becoming detached before developing epibasidia, 19–40 × 5–8 μ; spores (16–)20–22(–24) × (4–)5–6.5 μ; on coniferous wood .....14. *P. pustulata*

o. Basidia remaining attached, in corymbose clusters; spores less than 13 μ in length; on wood of *Lagerstroemia*.....p

p. Gregarious, the pustules anastomosing; thread-like paraphyses present; basidia up to 60 μ in length; spores 7.4–12.3 × 4–7.4 μ .....15. *P. lagerstroemiae*

p. Gregarious, but not crowded; paraphyses lacking; basidia 15–20 μ in length; spores 5–9 × 4–7 μ.....16. *P. caroliniana*

q. Spores 10 μ or less in length.....r

q. Spores 12 μ or greater in length.....t

r. Fructifications small, maculiform; hymenial elements other than basidia agglutinated; basidia 3-septate, 15–21 × 4–5 μ; epibasidia spreading at wide angles to the basidial axis; spores 4.5–6 × 4 μ; on *Populus* wood.....17. *P. micra*

r. Fructifications extensive; hymenial elements remaining distinct; epibasidia ascending.....s

s. Whitish; basidia 60–90 × 4–6 μ; spores ovate, 7–10 × 5–7 μ; clamp-connections lacking.....18. *P. effusa*

s. Avellaneous to brown; basidia 25–30 × 6–8 μ; spores subglobose to tear-shaped, 7–8 × 5.5–7 μ; clamp-connections abundant.....19. *P. sphaerospora*

t. Fructifications waxy; ochraceous; basidia emergent, the protruding portion 40–50 μ long, appressed to the surface; spores 11–14 × 7.5–9 μ.....20. *P. decipiens*

t. Fructifications gelatinous; whitish; basidia not emergent; spores mostly greater than 14 μ in length.....u

u. Fructifications 1–2 mm in diameter; gloeocystidia lacking; basidia 100–130 × 8–10 μ; spores 30 × 8 μ; on wood of *Cissus rhombifolia*.....21. *P. cissi*

u. Fructifications extensive; gloeocystidia or similar structure present.....v

v. Basidia without distinct hypobasidia, 1–3-septate, 40–50 × 9–10 μ; spores 15–30 × 5–9 μ.....22. *P. vestita*

v. Basidia with distinct, clavate hypobasidia, 3-septate, 51–91 × 5–7.8 μ; spores 13.5–21.6 × 5.8–9 μ.....23. *P. subvestita*

1. P. MIEDZYRZECENSIS Bres. Ann. Mycol. **1**: 113. 1903.

Fructification soft-gelatinous, almost mucoid, pulvinate, rounded or oblong, 3–6 mm in diameter, pale whitish to opalescent when moist, drying to an inconspicuous shining grey film; hyphae 1–3 μ in diameter with numerous clamp-connections; basidia 120–300 × 4–6 μ, flexuous,

finally almost circinate, 3-septate; epibasidia thick, becoming filiform; spores obovate or oblong, sometimes subfusiform, attenuate at the base and often laterally depressed, 8–16 × 5–8 μ, germinating by repetition.

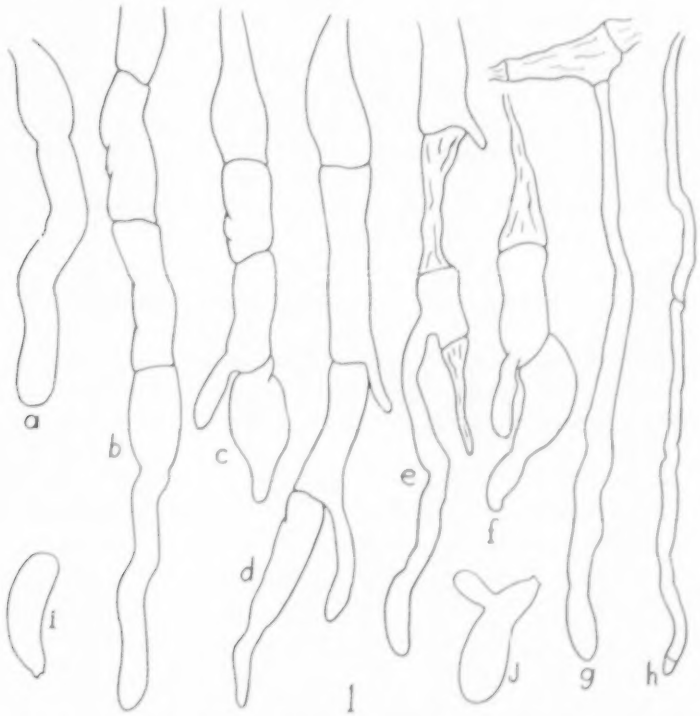
TYPE LOCALITY: Poland.

HABITAT: Attacking sphaeriaceous fungi on dead wood.

DISTRIBUTION: Georgia; Poland, France.

ILLUSTRATIONS: Bresadola, Ann. Mycol. **1**, pl. 3, f. 3; Olive, Mycologia **39**: 92, f. 1.

The above description is adapted from that given by Bourdot & Galzin (**1928**). Olive (**1947**) found this species growing from the



All figures drawn with the aid of a camera lucida and reduced in reproduction. Fig. 1, approximately × 750; Figs. 2–9, approximately × 1000.

FIG. 1. *Platygloea disciformis* (Fries) Neuhoff. a. Probasidium; b–f. Basidia at different stages of development; g. Epibasidium with collapsed basidial cell; h. Paraphysis; i, j. Basidiospores, one germinating.

perithecial stromata of a species of *Botryosphaeria* in Georgia and upon examining Bresadola's type, found that that is also growing over a sphaeriaceous fungus. Olive gives the basidial measurements as 97–268 × 5.1–7.2 μ, and the spore size as 9.1–12 × 5.9–8.1 μ. Bresadola's figures for the basidia are 75–200 × 4–6 μ and 10–13 × 7–9 μ for the spores.

2. P. ARRHYTIDIAE Olive, Bull. Torrey Bot. Cl. **78**: 103. 1951.
Platygloea fusco-atra Jacks. & Martin, Mycologia **32**: 691. 1940, p.p. (nom. conf.).

Distinct fructifications lacking, hyphae penetrating the basidiocarps of dacrymycetaceous fungi and producing basidia and conidia at the surface of the host; probasidia produced singly or in small apical or sub-apical clusters on the hyphal tips; basidia 2–4-septate, usually with distinct hypobasidia, the sporogenous portion 22–34 × 3.4–4.5 μ, often borne asymmetrically on the hypobasidium; hypobasidia clavate to pyriform, 7.5–16.5 × 4.5–6.8 μ; epibasidia broad at the base, 1–2 μ in diameter, variable in length; basidiospores ovoid, obovate, or short-cylindric, adaxially flattened, 7.5–10.5 × 3–4.9 μ, germinating by repetition; conidiophores short, broad, simple, in clusters; conidia acrogenous, subsphaerical to ovoid, 3–5.3 × 2.3–3.7 μ.

TYPE LOCALITY: Highlands, North Carolina.

HABITAT: Growing within the fructifications of dacrymycetaceous fungi.

DISTRIBUTION: Iowa, North Carolina, Ontario.

ILLUSTRATIONS: Olive, Bull. Torrey Bot. Cl. **78**: 104, f. 1–10; Martin, Mycologia **32**: 689, f. 6, a–c.

3. P. PENIOPHORAE Bourd. & Galz. Bull. Soc. Myc. Fr. **25**: 17. 1909.
Corticium ferax Ell. & Everh. Am. Nat. **31**: 339. 1896, p.p. (nom. conf.).

Waxy, thin, at first orbicular, 1–3 mm in diameter, becoming horny when dry, grey or brownish; hyphae 1–2 μ in diameter, with clamp-connections; basidia erect to flexuous, (20–)35–60 × (3–)4–7 μ, mostly 3-septate; epibasidia up to 90 μ long, filiform; spores hyaline, ovate, adaxially flattened, 5–7(–10) × 3.5–4(–6) μ; germinating by repetition; hymenial conidiophores branched, conidia 5–10(–12) × 3–4.5 μ.

TYPE LOCALITY: Allier, France.

HABITAT: Parasitic on various basidiomycetes.

DISTRIBUTION: North America; Europe.

ILLUSTRATIONS: Bourd. & Galz. Hymén. France 14, f. 7; Christiansson, Friesia **4**: 90; Martin, Mycologia **32**: 689, f. 5; Olive, Mycologia **39**: 92, f. 3, Jour. Mitchell Soc. **62**: pl. 11, f. 1–9; Wakefield and Pearson, Trans. Brit. Myc. Soc. **8**: 219, f. 5.

This species appears to be rather variable, as has been noted by Martin (**1940**) and Olive (**1947**). If the structure of the hymenial conidiophores and conidia is of any significance, then it should probably be divided into two or more species. It has been reported as parasitizing *Corticium, Peniophora, Poria, Odontia* and *Dacrymyces*. On gelatinous hosts it does not form a definite fructification, and is then distinguished from *P. arrhytidiae* by the conidial characters and by the lack of distinct hypobasidia. Three varieties have been described by Bourdot & Galzin (**1928**) and Olive (**1954**) described a fourth.

4. P. JAVANICA Pat. Bull. Soc. Myc. Fr. **14**: 190. 1898.

Gelatinous, erect, foliose-lobate, convoluted, 12–15 mm tall, brown; hymenium amphigenous; probasidia irregularly clavate, often toruloid; basidia cylindrical, about 50 × 6–8 μ, 3-septate, the apex obtuse, attenuate at the base; epibasidia long; spores ellipsoid to lenticular, apiculate, 10–13 × 6–8 μ, germinating by repetition or by the production of conidia.

TYPE LOCALITY: Java.

HABITAT: Dead branches.

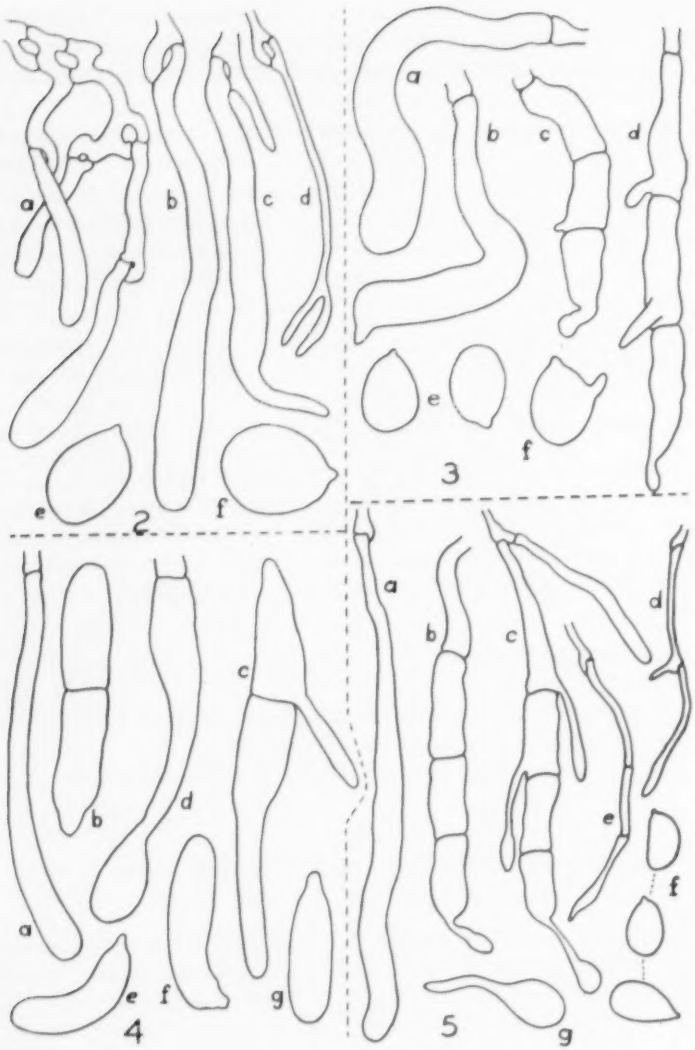
DISTRIBUTION: Known only from the type collection.

ILLUSTRATION: Patouillard, Tax. Hymén. 14, f. 7.

The above description is adapted from the description and illustration of Patouillard.

5. P. BRUNNEA (Martin) Martin, Lloydia **11**: 119. 1948.
Tjibodasia brunnea Martin, Lloydia **4**: 262. 1941.

Fleshy to sub-gelatinous, pezizoid, up to 1 cm in diameter, becoming larger by anastomosis, centrally attached and appressed against the substratum, white when fresh, drying dark brown; in section, thin at the margins, thick at the center, often with 2–3 superimposed hymenial layers, the outermost about 75 μ thick; paraphyses long-clavate, thick-walled; basidia cylindrical-clavate, (50–)67–70(–75) × (4–)5–6 μ, 3-septate; epibasidia cylindrical, 1.2–2.5 μ in diameter, long, often tortuous; basidiospores elliptical and adaxially flattened to broadly oval, (7–)8–9 × 4–5(–6) μ, germinating by repetition.



FIGS. 2–5.

FIGS. 2–5. 2. *Platygloea unispora* Olive. a. Hypha bearing three young probasidia; b. Mature probasidium; c. Basidium with epibasidium; d. Paraphysis; e, f. Basidiospores. 3. *Platygloea decipiens* Martin. a–d. Stages in basidial development; e, f. Basidiospores, one germinating. 4. *Platygloea pustulata* Martin and

TYPE LOCALITY: São Leopoldo, Brazil.

HABITAT: Dead wood.

DISTRIBUTION: Known only from the type locality.

ILLUSTRATIONS: Martin, Lloydia **4**: 264, f. 1; p. 268, f. 3–9.

The above description is adapted from that of Martin.

6. P. CARNEA Pat. Bull. Soc. Myc. Fr. **9**: 138. 1893.

Gelatinous, orbicular, effused, plano-convex, sessile, attached by a point, 5 mm in diameter, margin sinuous, pale rosaceous-yellow; hymenium venose; paraphyses long, slender; probasidia cylindrical; basidia 3-septate, elongate, 90 × 5 μ; epibasidia short, 5–12 μ in length; spores ovoid, hyaline, granular within, 10 × 5 μ, germinating by repetition.

TYPE LOCALITY: Pichincha, Ecuador.

HABITAT: Twigs and canes.

DISTRIBUTION: Mexico, Ecuador.

ILLUSTRATIONS: Patouillard, Bull. Soc. Myc. Fr. **9**: pl. 8, f. 4; Tax. Hymén. 14, f. 6.

This description is adapted from the description and illustrations by Patouillard. Martin (**1948**) referred to this species a collection from Mexico which appears to be very close. It differs in having shorter basidia than those described by Patouillard, and in having branched parapyhses which are septate, with obscure clamp-connections.

7. P. SUCCINEA Pat. Bull. Soc. Myc. Fr. **9**: 137. 1893.

Fructification soft-coriaceous, circular, attached at a single central point, applanate, 1 cm in diameter, 1 mm thick, margins free, more or less sinuate, golden yellow, translucent; hymenium in vein-like folds; probasidia clavate; basidia cylindric, 2–3-septate, constricted at the septa, 40–50 × 6 μ; epibasidia filiform, 15–20 μ in length; spores ovoid, smooth, hyaline, 10–12 × 8 μ, germinating by repetition.

TYPE LOCALITY: Pululahua, Ecuador.

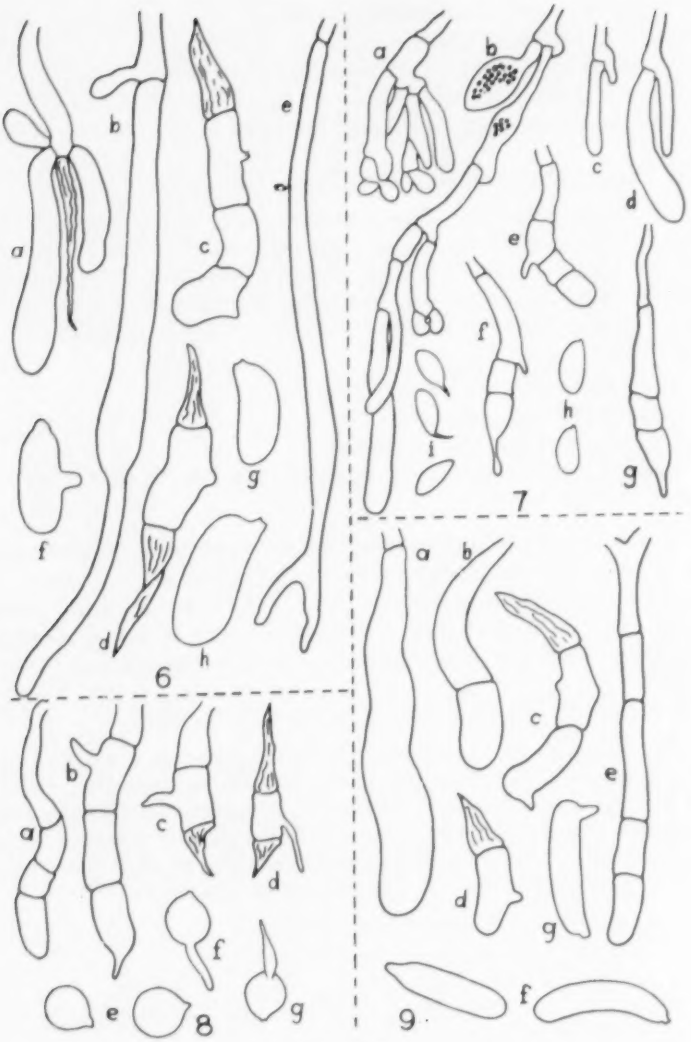
HABITAT: Dead branches of *Melastoma*.

DISTRIBUTION: Ecuador.

ILLUSTRATIONS: Patouillard, Bull. Soc. Myc. Fr. **9**: 18, f. 2.

The above description is based on the illustrations and description of Patouillard.

Cain. a. Probasidium; b. Young basidium; c. Two-celled basidium; d. Single-celled basidium; e–g. Basidiospores. 5. *Platygloea carnea* Pat. a. Probasidium; b, c. Basidia; d, e. Paraphyses; f. Three basidiospores; g. Germinating basidiospore.



FIGS. 6–9.

FIGS. 6–9. 6. *Platygloea subvestita* Olive. a. Cluster of young probasidia; b. Probasidium showing clavate hypobasidium and cylindrical sporogenous portion; c, d. Portions of mature basidia; e. Glococystidium; f–h. Basidiospores, one germinating. 7. *Platygloea peniophorae* Bourd. & Galz. a. Hyphae bearing conidiophores; b. Hypha with peculiar "gall cells" basally and bearing a conidiophore and two young probasidia; c, d. Probasidia; e–g. Basidia; h. Basidiospores;

8. P. FIMETARIA (Schum. ex Pers.) Höhnel, Ann. Mycol. **15**: 293. 1917.

Tremella fimetaria Schum. ex Pers. Myc. Eur. **1**: 105. 1822.
Platygloea fimicola Schroet. Krypt.-Fl. Schles. **3**[1]: 384. 1887.
Helicobasidium fimetarium Boud. Jour. de Bot. **1**: 330. 1887.
Achroomyces fimicola (Schroet.) Migula, Krypt.-Fl. Deutschl. **3**[2]: 1. 1912.

Waxy-membraneous, discoid, plane or slightly concave, 2–4 mm in diameter, pale flesh color to pale violet; hyphae slender, much-branched; basidia long-cylindric, 36–42 × 5–6 μ, 3-septate; epibasidia awl-shaped or thread-shaped, up to 11 μ long; spores hyaline, ovoid, 11 × 7 μ.

TYPE LOCALITY: Germany.

HABITAT: Dung of various herbivores.

DISTRIBUTION: Manitoba; Europe.

ILLUSTRATIONS: Boudier, Jour. de Bot. **1**: 331, f. a–k; Martin, Univ. Iowa Stud. Nat. Hist. **18**: 79, f. 28; **19**: 111, f. 28.

The above description is adapted from that by Schroeter. I have examined notes and drawings by Gladys E. Baker taken from a Canadian collection lent her by G. R. Bisby. The basidia, as figured by her, have a definite hypobasidium, more or less clavate in shape and about 14–25 × 5–7 μ in size. In addition, she noted the presence of clamp-connections, and a slightly smaller spore size (10 × 4–5 μ). Boudier lists the spore size as 9–11 × 4–8 μ, and the basidia in his illustrations do not show any evidence of distinct hypobasidia.

9. P. DISCIFORMIS (Fries) Neuhoff, Ark. for Bot. **28A**(1): 56. 1936.

Tremella disciformis Fries, Syst. Myc. **2**: 216. 1823.
?*Agyrium nigricans* var. *minus* Fries, Syst. Myc. **2**: 233. 1823.
Stictis tiliae Lasch. Bot. Zeit. **3**: 66. 1845.
?*Achroomyces tumidus* Bonorden, Hand. allg. Myc. 135. 1851.
Platygloea nigricans Schroet. Pilze Schles. **3**[1]: 384. 1887.
Tachaphantium tiliae Bref. Unters. **7**: 79. 1888.
Platygloea tiliae (Bref.) Sacc. Syll. Fung. **6**: 771. 1888.
Achroomyces tiliae (Bref.) Höhnel, Ann. Mycol. **2**: 271. 1904.
?*Ocellaria betuli* var. *nigrescens* (Fries) Rehm, Hist. und Discomycet., 136. 1896.

i. Conidia. 8. *Platygloea sphaerospora* Martin. a. Mature probasidium; b. Basidium; c, d. Basidial cells; e–g. Basidiospores, one germinating by repetition, another by germ tube. 9. *Platygloea vestita* Bourd. & Galz. a, b. Probasidia; c, d. Basidial cells; e. Gloeocystidium-like structure; f, g. Basidiospores, one germinating by repetition.

Fructifications soft, waxy or gelatinous in texture, breaking through the bark as subrotund, flattened disks, 1.5–2 mm in diameter, about 1–1.5 mm in height, smooth, sordid white to pale flesh-color, becoming pruinose, cinnamon-brown to black and slightly wrinkled; hymenium composed of large irregular basidia and many-branched septate, thread-like paraphyses; basidia mostly 3-septate, 90–180(–300) × 9–12 μ, with distinct, thin-walled hypobasidia; epibasidia long, tortuous, up to 90 × 6 μ, and occasionally cut off from the basidium by a septum; spores cylindrical to allantoid, 26–30(–36) × (6–)7.5–9 μ.

TYPE LOCALITY: Sweden.

HABITAT: Dead branches of birch and linden.

DISTRIBUTION: Germany, France, Sweden.

ILLUSTRATIONS: ?Bonorden, Handb. Allg. Myc. 135, pl. 11, f. 231; Bourdot & Galzin, Hymén. Fr. 14, f. 9; Brefeld, Unters. **7**, pl. 4, f. 12–14; Lindau, in Engl. & Prant. Nat. Pflanzenf. 1^1: 85, f. 56, G, H; Migula, Krypt.-Fl. Deutschl. 3^2: 2, f. 1, 2; Neuhoff, Bot. Arch. **8**: pl. 1, f. 14; Olive, Bull. Torrey Bot. Cl. **78**: 106, f. 19–26; Riess, Bot. Zeit. **11**: 135, pl. 3, f. 21–23.

10. P. FIBROSA Pat. Bull. Soc. Myc. Fr. **38**: 84. 1922.

Receptacle firm, gelatinous, orbicular or elliptic, sessile, applanate, expanded, 2–4 cm in diameter, brownish yellow, horny when dry, with free margins surrounded by white or pale yellowish fibrillose mycelium; paraphyses abundant, linear, colorless, 3 μ in diameter; basidia cylindrical, about 75 × 5 μ, 3-septate, the lower cell narrowed into a long filament immersed in the basal portion of the fructification; basidiospores ovoid, somewhat inequilateral, smooth, hyaline, 12–15 × 6 μ.

TYPE LOCALITY: Los Baños, Philippine Isl.

HABITAT: Dead wood of *Lansium domesticum*.

DISTRIBUTION: Known only from the type locality.

The above description is adapted from the original.

11. P. UNISPORA Olive, Jour. Mitchell Soc. **60**: 17. 1944.

Waxy, pustulate, the pustules subspherical, mostly less than 0.5 mm in diameter, gregarious or scattered, usually remaining distinct, yellow to brown when moist, drying to cinnamon or dark brown; paraphyses scarce, branched, with basal clamp-connections, abortive branched basidia also present; probasidia long, cylindrical-clavate, each with a conspicuous basal clamp-connection; basidia 61–83 × 6.1–7.6 μ, cylindrical, non- (or 1–3-) septate, each with a single terminal epibasidium; epi-

basidia broad at the base, tapering, up to 15 μ in length; spores subspherical to broadly oval, inequilateral, 12.8–18.3 × 9.6–13.7 μ.

TYPE LOCALITY: Chapel Hill, North Carolina.
HABITAT: Dead branches of *Juniperus*.
DISTRIBUTION: North Carolina, Georgia.
ILLUSTRATIONS: Olive, Jour. Mitchell Sc. Soc. **60**: pl. 2, f. 3; pl. 4, f. 1–11; Mycologia **39**: 92, f. 2.

12. P. LONGIBASIDIA Lowy, Mycologia **46**: 100. 1954.

Fructifications gelatinous, pustulate, the pustules up to 6 mm broad, becoming confluent and effused, white, drying to a whitish or greyish-white conspicuous horny film; hyphae 3–4.5 μ in diameter, without clamp-connections, sparsely septate; paraphyses narrowly cylindrical, 2–3.5 μ in diameter, some emergent, extending beyond the surface of the fructification 55–100 μ or more, others terminating near the distal end of the basidia; probasidia clavate; basidia cylindrical, 0–4-septate, (38–)52–78(–135) × 5.5–9.6 μ; basidiospores hyaline, cylindrical to allantoid, apiculate, (12.5–)16.5–25.5(–31) × (5.5–)6–7.5(–8) μ, germinating by germ tube or repetition.

TYPE LOCALITY: Garwood, Louisiana.
HABITAT: On dead, partly decorticated wood.
DISTRIBUTION: Known only from the type locality.
ILLUSTRATIONS: Lowy, Mycologia **46**: 102–103, f. 1–14.
The above description is taken from the original diagnosis.

13. P. BLASTOMYCES Möller, Protobas. 29. 1895.

Waxy, pustulate, more or less cerebriform, up to 1 cm broad and 5 mm thick, greyish white with pale yellow tints; hyphae delicate, 2–3 μ in diameter, closely intertwined; basidia thread-like, up to 200 × 4 μ, mostly 3-septate; epibasidia thread-like, length variable; spores oval, 12 × 6 μ, germinating by repetition, budding, or germ tube.

TYPE LOCALITY: Blumenau, Brazil.
HABITAT: On decaying bark.
DISTRIBUTION: Brazil, Colombia.
ILLUSTRATIONS: Möller, Protobas. pl. 4, f. 5.
The above description is based on that of Möller.

14. P. PUSTULATA Martin & Cain, Mycologia **32**: 691. 1940.

Gelatinous, pustulate, the individual pustules 1–3 mm in diameter, anastomosing, white to greyish when moist, drying to an inconspicuous

horny film; hyphae radiating, septate, branched, about 2 μ in diameter, terminating in slender, branched paraphyses, 2–2.5 μ in diameter, or in long, clavate probasidia; basidia usually 1-septate, less commonly with 2 or 3 septa (or non-septate and producing a single spore), cylindrical, generally becoming detached before developing epibasidia, 19–40 × 5–8 μ; epibasidia 2–3(–4.5) μ in diameter, cylindrical; spores cylindrical-allantoid, often strongly curved (16–)20–22(–24) × (4–)5–6.5 μ, conspicuously apiculate.

TYPE LOCALITY: Duchesnay, Quebec.

HABITAT: On dead coniferous wood and bark.

DISTRIBUTION: Quebec, Ontario.

ILLUSTRATIONS: Martin, Mycologia **32**: 689, f. 7.

15. P. LAGERSTROEMIAE Coker, Jour. Mitchell Soc. **35**: 124. 1920.

Firm, waxy, pulvinate, gregarious, the individual pustules up to 1.5 mm broad, with indefinite margins, often anastomosing to form elongated, more or less nodulose, irregular patches, up to 1–2 cm in extent, the surface dull, granular, dull white or straw-colored, becoming sordid or smoky-brown; hymenium with numerous thread-like paraphyses; basidia in corymbose clusters, up to 20 μ or more in length by 5–5.5 μ thick, 1-septate, or with a slender stalk cell which collapses early, often strongly curved; epibasidia terminal, ascending; spores oval or elliptical, curved, 7.4–12.3 × 4–7.4 μ, germinating by repetition.

TYPE LOCALITY: Chapel Hill, North Carolina.

HABITAT: Dead branches of *Lagerstroemia*.

DISTRIBUTION: Known only from the type locality.

ILLUSTRATIONS: Coker, Jour. Mitchell Soc. **35**: pl. 41, f. 3; pl. 54, f. 8–14.

Differs from *P. caroliniana* by the presence of paraphyses, larger spores and basidia, and the crustose habit with indefinite margins. Coker does not give the basidial length, but his illustrations indicate a length of about 25–50 μ.

16. P. CAROLINIANA Coker, Jour. Mitchell Soc. **35**: 123. 1920.

Waxy, pulvinate, 0.5–2.5 mm in diameter, 0.5–1.5 mm high, gregarious but not crowded, dull smoky flesh-colored becoming sordid, surface granular, becoming convolute, finally collapsing; hyphae 2–3.7 μ in diameter, without clamp-connections, branching at open angles in the interior, in a densely corymbose manner near the surface and terminating in clusters of basidia; basidia 15–20 × 5.2–6.5 μ, swollen, not strongly curved, 1-septate or sometimes with a second septum cutting off a stalk-

like basal cell, spores sub-oval, smooth, yellowish or pinkish-yellow in mass, 5–9 × 4–7 μ, germinating by repetition.

TYPE LOCALITY: Chapel Hill, North Carolina.

HABITAT: Dead branches of *Lagerstroemia.*

DISTRIBUTION: Known only from the type locality.

ILLUSTRATIONS: Coker, Jour. Mitchell Soc. **35**: pl. 23, f. 13; pl. 54, f. 1–7.

This description, and the preceding one, are taken from those by Coker.

17. P. MICRA Bourd. & Galz. Bull. Soc. Myc. Fr. **39**: 261. 1924.

Mucoid, maculiform, scarcely spreading, subopalescent, becoming yellowish or brownish on drying; hyphae indistinct, agglutinated (0.5–2 μ); probasidia at first ovoid, then elongate; basidia straight or curved, 15–21 × 4–5 μ, 3-septate; epibasidia 9–12 × 2 μ, spreading at right angles to the basidial axis; spores oblong, 4.5–6 × 4 μ, germinating by repetition.

TYPE LOCALITY: Aveyron, France.

HABITAT: Dead *Populus* wood.

DISTRIBUTION: France.

ILLUSTRATIONS: Bourdot & Galzin, Hymén. Fr. 13, f. 8.

This description is adapted from that given by Bourdot & Galzin (**1929**). According to the authors, this species is distinguished by its granular texture, indistinct hymenial elements, and by the epibasidia spreading at right angles to the basidial axis.

18. P. EFFUSA Schroet. Krypt.-Fl. Schles. **3**[1]: 384. 1887.
Achroomyces effusa (Schroet.) Migula, Krypt.-Fl. von Deutsch. **3**[2]: 1. 1912.

Firm-gelatinous, effused, closely adherent, opalescent to yellowish, whitish when dry, the hymenium puberulent under the lens; subhymenial hyphae slender, 1–2 μ in diameter, arising erect and parallel from a compact basal stratum composed of somewhat broader hyphae (4–5 μ); basidia curved, almost circinate, 60–90 × 4–6 μ, 3-(4-)septate; spores hyaline, ovate, apiculate, 7–8(–10) × 5–7 μ, germinating by repetition.

TYPE LOCALITY: Germany.

HABITAT: On fallen branches.

DISTRIBUTION: Germany, France, England.

ILLUSTRATIONS: Wakefield and Pearson, Tr. Br. Myc. Soc. **6**: 138.

This description is based on that of Wakefield and Pearson (**1919**), and that of Bourdot and Galzin (**1909**). Wakefield and Pearson give smaller basidial measurements (40–50 × 4–5 μ) and spores of a smaller diameter (4–5 μ). Bourdot and Galzin distinguish between primary spores (7–10 × 5–7 μ) and those that are secondary (7–8 × 5–6 μ).

19. P. SPHAEROSPORA Martin, Mycologia **26**: 261. 1934.

Waxy to fleshy, broadly effused, separable, avellaneous to wood-brown, drying deep brownish-red; hyphae mostly thin 1.5–2(–5) μ, closely interwoven, with numerous clamp-connections; probasidia swollen, clavate, 25–30 × 6–8 μ; basidia cylindrical, 25–40 × 3.5–6 μ, 3-septate; spores ovoid to subglobose, some laterally flattened, 7–8 × 5.5–7 μ, germinating by repetition or by germ tube.

TYPE LOCALITY: Dias Creek, Cape May Co., New Jersey.
HABITAT: On rotten wood of *Quercus falcata*.
DISTRIBUTION: Known only from the type locality.
ILLUSTRATIONS: Martin, Mycologia **26**: 264, pl. 31, f. 1, 2.

20. P. DECIPIENS Martin, Lloydia **11**: 119. 1948.

Waxy, broadly effused, with mucedinoid reticulate margins, deep brownish-ochraceous, to dingy ochraceous, drying darker, horny, brittle; in section 150–225 μ thick, composed of 3 layers, the basal one of loosely arranged horizontal hyphae, 3.5–4 μ in diameter, giving rise to a dense intermediate stratum of intricately woven, gelatinized and metamorphosed hyphae with hymenial remnants, merging into the hymenium; clamp-connections lacking; probasidia cylindrical to clavate, protruding above the surface 40–50 μ, about 4.5–8.5 μ in diameter, often sharply bent and appressed to the surface, becoming 2- to 3-septate; basidia cylindrical, bent, 40–60 × 4.5–7 μ; epibasidia short, broad; basidiospores ovoid, inequilateral, 11–14 × 7.5–9 μ, germinating by germ tube or by repetition.

TYPE LOCALITY: Prov. Chiriquí, Panamá.
HABITAT: Dead wood.
DISTRIBUTION: Known only from the type collection.
ILLUSTRATIONS: Martin, Lloydia **11**: 115, f. 3.

20. P. CISSI Pat. Bull. Soc. Myc. Fr. **9**: 137. 1893.

Fructifications gelatinous, effused, applanate, 1–2 mm in diameter, scattered, superficial, white; context of slender interwoven hyaline

hyphae, 2–3 μ in diameter; basidia cylindrical, obtuse at the apex, attenuate below, 100–130 × 8–10 μ, 3-septate, constricted at the septa; epibasidia elongate, filiform; spores hyaline, granular within, reniform, 30 × 8 μ, germinating by repetition.

TYPE LOCALITY: Pululahua, Ecuador.
HABITAT: Dead branches of *Cissus rhombifolia*.
DISTRIBUTION: Known only from the type collection.
ILLUSTRATIONS: Patouillard, Bull. Soc. Myc. Fr. **9**: pl. 8, f. 3.
The above description is adapted from that of Patouillard.

22. P. VESTITA Bourd. & Galz. Bull. Soc. Myc. Fr. **39**: 261. 1923.

Mucoid-gelatinous, effused, thick, greyish or hyaline, almost invisible when dry, clothed with a thin white felty layer; hyphae 8–10 μ in diameter, without clamp-connections, producing erect branches, flexuous and distinguished by irregular thickenings, 60–100 × 6–10 μ, projecting above the surface 40–50 μ; probasidia at first ovoid, then cylindrical, becoming 1–3-septate; basidia 40–50 × 9–10 μ, curved; epibasidia at first conical, becoming slender, up to 20 μ in length, spores oblong-ellipsoidal or subcylindrical, obliquely attenuate at the base and laterally depressed, 15–21–30 × 5–7.5–9 μ.

TYPE LOCALITY: Aveyron, France.
HABITAT: On dead branches and litter.
DISTRIBUTION: Iowa; France, England.
ILLUSTRATIONS: Bourdot & Galzin, Hymén. France, 14, f. 10.
The above description is essentially the same as that given by Bourdot and Galzin. The Iowa specimen has smaller basidia, 30–50 × 5–6.5 μ, and spores mostly 18–21 × 5–7 μ, germinating by repetition.

23. P. SUBVESTITA Olive, Bull. Torrey Bot. Club **80**: 33. 1953.

Fructification soft-gelatinous to soft waxy-gelatinous, broadly effused, thin, surface tuberculate or nearly smooth, sordid hyaline to tan, drying to an invisible film; hyphae without clamp-connections; gleocystidia clavate to cylindric, sometimes much elongated, simple or branched, frequently irregular, 26.4–210 × 4.7–10.9 μ, contents finely granular to viscous, yellowish; probasidia arising terminally on the hyphae, frequently several in a group, consisting of a clavate hypobasidial portion 38.8–63.6 × 7–10.9 μ, and an apical cylindric 4-celled sporogenous portion; entire basidia measuring 51–91 × 5–7.8 μ; epibasia short to elongate; basidiospores allantoid, apiculate, 13.5–21.6 × 5.8–9 μ, germinating by repetition or by germ tube.

TYPE LOCALITY: Highlands, North Carolina.

HABITAT: Decorticate frondose wood.

DISTRIBUTION: Known only from the type locality.

ILLUSTRATIONS: Olive, Torrey Bot. Cl. Bull. **80**: 34, f. 1–11, p. 37, f. 18.

The above description is adapted from that of Olive (**1953**). I found that some of the basidia were well over 100 μ in length and two of Olive's figures (p. 34, f. 2, 4) are scaled to indicate basidial sizes of 150–200 μ.

EXCLUDED SPECIES

Platygloea criophori (Bres.) Höhn. Sitz. Akad. Wiss. Wien. M.-N. Kl. I. **118**: 1159. 1909, = *Xenogloea criophori* (Bres.) H. & P. Syd. Ann. Mycol. **17**: 44. 1919.

P. fusco-atra Jacks. & Martin, Mycologia **32**: 691. 1940 = nomen confusum.

The type consists of an immature dacrymycetaceous fungus, possibly *Arrhytidia enata*, parasitized by *Platygloea arrhytidiae*.

P. grandinia Rick, Egatea **18**: 211. 1933, = *Patouillardina cinerea*.

P. hymenolepidis Racib. Bull. Int. Sci. Cracow. Nat. Cl. **1909**: 356.

The coiled intracellular haustoria and parasitism of ferns indicate that this species should be transferred to some other genus, e.g., *Herpobasidium* or *Platycarpa*.

P. lagerheimii Sacc. & Syd., = *Helicogloea lagerheimii* Pat.

P. minima Rick, Egatea **18**: 211. 1933.

The description given by Rick is so inadequate as to make it impossible to place this species in the key. His description is as follows: Hyaline, gelatinous, scarcely 1 mm in diameter; basidia and epibasidia long, filiform; spores pyriform, 7 × 3 μ. Collected in Brazil on a polyporaceous fungus.

P. pini Höhn. Verh. Zool.-Bot. Ges. Wien. **60**: 331. 1910, is a *nomen nudum*.

Tachaphantium sp.

Gilbert (**1910**) described a specimen collected in Wisconsin which seems to be distinct, but to which he assigned no name.

Tjibodasia pezizoides Holterm. Myc. Unters. Trop. 44. 1898.

Description inadequate (see Martin, Lloydia **11**: 119. 1948).

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MARINE FUNGI. II. ASCOMYCETES AND DEUTEROMYCETES FROM SUBMERGED WOOD

T. W. JOHNSON, JR.[1]

(WITH 43 FIGURES)

During the spring and summer of 1955, a preliminary study was made of marine fungi in the Beaufort Inlet region, Beaufort-Morehead City, North Carolina. Marine and brackish-water species of *Leptosphaeria* and *Pleospora* were described from this area in a previous paper. Other Ascomycetes and some Fungi Imperfecti are reported in this paper, together with notes on trapping methods.

Barghoorn and Linder (**1944**) "trapped" for marine fungi by attaching wood panels directly to pilings. Meyers (**1953**) devised a "sandwich" trap by attaching two wood panels to lengths of brass sash-weight chain so that the inner faces of the panels were separated by the width of the chain links. These investigators also recommended the examination of driftwood for marine fungi.

Several modifications of the sandwich traps were tested in the Beaufort Inlet. The basic trap pattern consisted of 4″ × 6″ × 1/28″ veneer panels. These were attached in series of 2–6 and separated by oak strips to give distances between the panels of 1/16, 1/8, 1/4, and 3/4 inch. Veneers of white oak, birch, cherry, gum, mahogany, yellow poplar, ash, chestnut, prima vera, and cedrillo were used. Multiple traps of 4″ × 6″ × 3/8″ pine panels were also tested. Traps were attached to brass chain and submerged at the entrance to estuaries and in open water for periods of 2–36 weeks.

Fouling organisms, algae, bryozoans, and barnacles, in particular, made examination of the traps very difficult. Careful scraping of the encrusting organisms from the panel surfaces was the only means of exposing the underlying wood. Obviously, some fungi must inadvertently have been destroyed or were overlooked.

[1] Funds, from the Duke University Council on Research, to defray a portion of the expense of this investigation, are gratefully acknowledged. I am indebted to Professor Leland Shanor and to Mr. S. P. Meyers, for reviewing the manuscript. Dr. F. A. Wolf very kindly provided the Latin diagnoses and assisted in several of the taxonomic problems.

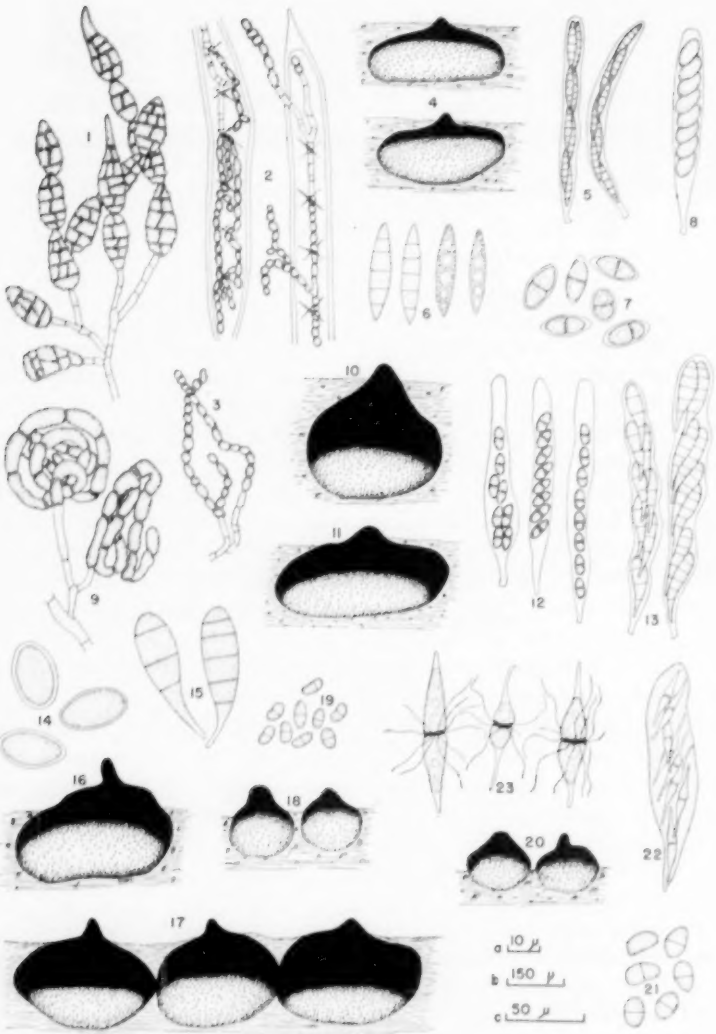


FIG. 1. *Alternaria maritima*. Conidia and portion of conidiophore. FIGS. 2, 3. *Hormiscium* sp. Conidia and vegetative hyphae. FIGS. 4–6. *Sphaerulina* sp. 4. Perithecia. 5. Asci. 6. Mature and immature ascospores. FIGS. 7, 10–12. *Massariella maritima*. 7. Ascospores. 10, 11. Perithecia. 12. Asci. FIGS. 8, 14, 17. *Physalospora* sp. 8. Ascus. 14. Ascospores showing gelatinous sheath. 17. Perithecia. FIG. 9. *Helicoma salinum*. Conidia and branched conidiophore.

The rate of water movement between two panels is slower than that around the exposed faces. Hence it may be assumed that fungus spores would become attached more readily to the inner than to the outer surface of two facing panels. Counts of fructifications on the outer and inner surfaces of 12 traps were 11–24 per cent higher on the latter. These figures may not, however, represent the actual numbers present since the number of encrusting organisms (colonies or individuals) on the inner surfaces was about 30 percent greater than on the outer panel surfaces. The inner faces of "sandwich" traps are not only more suitable for fungi, but also for other organisms. Thus, such traps are probably not the most efficient for such work.

As a consequence of the high percentage of wood surface covered by fouling organisms, no comparative figures are available on the influence of type of wood on numbers and kinds of fungi trapped. More suitable traps must be devised before such estimates can be made. The fungi reported in this paper, therefore, probably represent only a small portion of the actual fungal population.

With the exception of *Stemphylium maritimum*, the following fungi are described as they appeared on the substratum. Some entities are described without specific names being assigned to them. I believe this is justified in view of either paucity of material or where attempts at obtaining pure cultures were unsuccessful.

DEUTEROMYCETES

ALTERNARIA MARITIMA Sutherland, New Phytol. **15**: 46. 1916. (FIG. 1). On birch veneer, submerged 11 weeks. Except for larger conidial size, the Beaufort material is similar to the verrucose form described by Sutherland. Linder's form (Barghoorn & Linder, **1944**) produced both smooth and verrucose conidia.

HORMISCIUM sp. (FIGS. 2, 3). Mycelium extensive. Vegetative hyphae hyaline, developing into catenulate, ovoid or spherical, light brown conidia. Conidia 6–13 × 5–9 μ. Conidia and vegetative hyphae superficial or innate in wood elements.

Substratum: birch veneer, submerged 8 weeks, Pivers Island, Beaufort, North Carolina, December 11, 1955.

FIGS. 13, 15, 16. *Sphaerulina pedicellata*. 13. Asci. 15. Ascospores. 16. Perithecium. FIGS. 18–21. *Diplodia orae-maris*. 18. Pycnidia of large form. 19. Conidia of typical form. 20. Pycnidia of typical form. 21. Conidia of large form. FIGS. 22, 23. *Peritrichospora integra*. 22. Ascus with spore initials. 23. Ascospores.

Figs. 1–3, 5, 8, 9, 12, 13, 20, 22, scale c; figs. 4, 10, 11, 16–18, scale b; others, scale a.

Repeated attempts to culture the fungus failed. Little is known of its variability hence the species cannot be identified with certainty. This is apparently the first report of a marine representative of the genus.

HELICOMA SALINUM Linder, *In* Barghoorn & Linder, Farlowia **1**: 406. 1944. (FIG. 9). On yellow poplar veneer, submerged 9 weeks. The coiled conidia are predominantly 55–68 μ in diameter.

DIPLODIA ORAE-MARIS Linder, *In* Barghoorn & Linder, Farlowia **1**: 403. 1944. (FIGS. 18–21). Two forms were collected on mahogany and yellow poplar traps submerged for 12 weeks. One form (FIGS. 19, 20) produced conidia nearly the same size as those described for the species by Linder, that is, 6–7.5 × 4–5 μ. The second form (FIGS. 18, 21) had slightly larger conidia (8–11 × 5–7.5 μ). Previously reported from Long Beach, California.

Stemphylium maritimum sp. nov. FIGS. 39–41

Hyphis vegetis pallido-flavis, septatis. Conidiophoris flavo-brunneis vel fusceis, septatis, quisque cellula 2–4-guttulata, apiculata, inflatione terminali deficiente. Conidiis singulis, fuscis, globosis vel ellipsoideis vel pyramidatis vel irregularibus vel apiculatis, muriformibus vel sarciniformibus, levibus vel verruculosis, 19–32 × 17–29 μ.

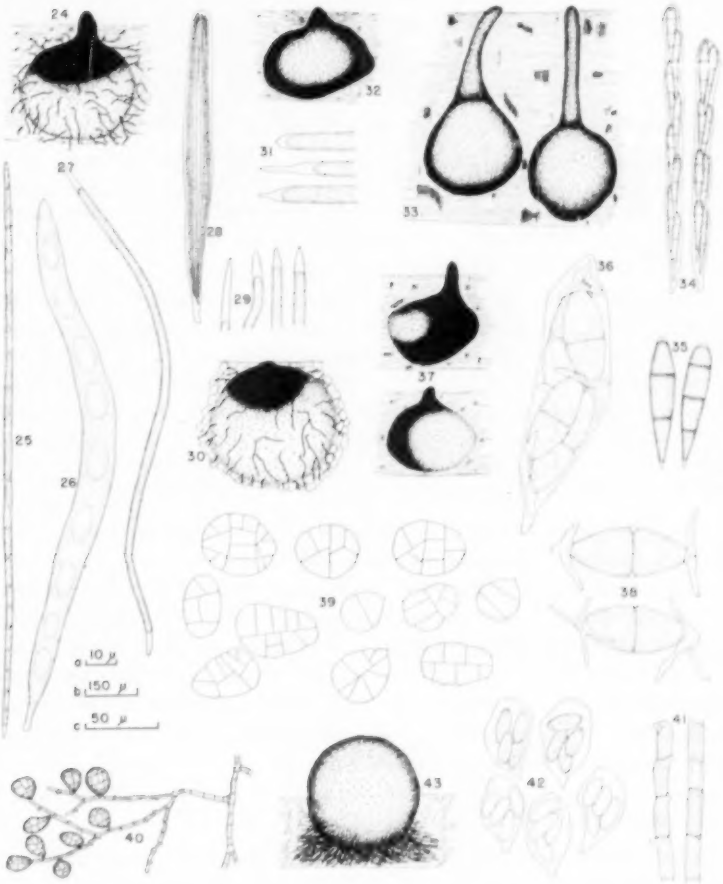
Vegetative hyphae light yellow, septate, cells containing 2–4 rows of guttules. Conidiophores erect, yellow-brown at base, dark brown at apex; terminal swelling lacking, replaced by a short, lateral apiculus from each cell. Conidia dark brown, globose, ellipsoid, pyramidal, irregular, or short-apiculate; muriform, occasionally sarciniform; single, smooth or verrucose, 19–32 × 17–29 μ.

On pine panel, submerged 6½ months, Beaufort Inlet Channel, Beaufort, North Carolina, January 2, 1956, TYPE.

Wiltshire (**1938**) recommended expansion of the generic limits of *Stemphylium* to include *S. botryosum* sensu Oudemans, and *S. lanuginosum* Harz. With this expanded concept, there is no doubt that the foregoing fungus is a *Stemphylium*. The fungus was isolated on sea water agar and transferred to potato-dextrose agar. It grows as well on fresh-water agars as on sea water agar.

Stemphylium maritimum is very near *S. botryosum*, but the conidia of the marine form are more globose. Furthermore, the apiculate conidiophore cells (FIG. 41) are lacking in *S. botryosum*. *Stemphylium maritimum* is suggestive of *S. sarcinaeforme* only in conidial septation. *Stemphylium codii* (Zeller, **1918**) differs from *S. maritimum* in that the conidia are larger, not verrucose, and often occur on short pedicels.

SPEIRA PELAGICA Linder, *In* Barghoorn & Linder, Farlowia **1**: 407.



FIGS. 24–32. *Lulworthia*. *24–26*, 30, 31. *L. medusa*. *24*. Perithecium in wood. *25*. Ascospore. *26*. Immature ascus. *30*. Perithecium in *Spartina* stem. *31*. Ascospore tips. *27–29*, *32*. *L. salina*. *27*. Ascospore. *28*. Ascus. *29*. Ascospore tips. *32*. Perithecium. FIGS. 33–35. *Ceratosphaeria* sp. *33*. Perithecia. *34*. Asci and spores. *35*. Ascospores. FIGS. *36–38*. *Remispora maritima*. *36*. Ascus with spores. *37*. Perithecia. *38*. Ascospores. FIGS. *39–41*. *Stemphylium maritimum*. *39*. Conidia. *40*. Conidia and conidiophore. *41*. Cells of conidiophore and vegetative hypha. FIGS. *42*, *43*. Plectomycete. *42*. Asci and spores. *43*. Superficial ascocarp.

Figs. 24, 30, 32, 33, 37, scale b; figs. 25, 26, 28, 34, 40, 43, scale c; others, scale a.

1944. On yellow poplar veneer, submerged 9 weeks. The conidia nearly always consist of 2–5 rows of cells.

ASCOMYCETES

Massariella maritima sp. nov. FIGS. 7, 10–12

Peritheciis innatis, ostiolis brevibus ac lato-conicis, nigris, 380–600 μ diam., 216–264 μ altis. Ascis cylindraceis vel clavatis, 8-sporis, 96–152 × 8–24 μ, plerumque 128–144 × 8–10 μ. Ascosporis ovatis vel ellipsoideis, flavo-brunneis, 1-septatis, constrictis, mucoinvolutis, uniseriatis vel partim biseriatis, 10–15 × 5–10 μ, plerumque 11–14 × 5–7 μ. Paraphysatis.

Perithecia scattered, innate except for the short, broad-conic ostioles, black, 380–600 μ in diameter, 216–264 μ high. Asci cylindrical or clavate, occasionally elongate-naviculate; thin-walled, 8-spored, 96–152 × 8–24 μ, predominantly 128–144 × 8–10 μ. Ascospores ovate to elongate-ellipsoid, or oblong-ellipsoid, yellow-brown, uniseptate, constricted at the septum; each cell uniguttulate; surrounded by a gelatinous sheath; uniseriate to partially biseriate, 10–15 × 5–10 μ, predominantly 11–14 × 5–7 μ. Paraphyses filiform, simple or sparingly branched.

On pine panel, submerged 3 months, off Fort Macon, Bogue Banks, Beaufort, North Carolina, August 10, 1955, TYPE.

The species is characterized by the uniseptate, yellow-brown ascospores surrounded by a conspicuous sheath (FIG. 7). There may be some question of the generic position of the fungus since it might equally well be placed in *Massaria*. Most species of *Massaria* have pluriseptate spores, or, if 1-septate, they tend toward being more septate. *Massariella* is limited to species with uniseptate spores only. This is apparently the first record of a marine species in the genus.

Sphaerulina pedicellata sp. nov. FIGS. 13, 15, 16

Peritheciis innatis, ostiolis brevibus ac papillatis, nigris superne ac brunneis inferne, membraneis, subglobosis vel ellipsoideis, 412–720 μ diam., 250–325 μ altis. Ascis cylindraceis vel clavatis, 8-sporis, 120–215 × 16–24 μ, interdum diffluentibus. Ascosporis elongato-ovoideis vel elongato-pyriformibus, una extremitate attenuatis ac curvulis, hyalinis, 3–5-septatis, partim vel perfecte biseriatis, 38–54 × 10–15 μ, plerumque 40–51 × 11–14 μ. Aparaphysatis.

Perithecia scattered, innate except for the short, papilliform ostioles, black above, brown below, membranous, subglobose or ellipsoid, 412–720 μ in diameter, 250–325 μ high. Asci cylindrical or clavate, straight or curved, thin-walled, 8-spored, 120–215 × 16–24 μ, occasionally deliquescing. Ascospores elongate-ovoid to elongate-pyriform, one end-cell attenuate and curved; hyaline, 3–5-septate, not constricted at septa,

densely guttulate, partially to completely biseriate, 38–54 × 10–15 μ, predominantly 40–51 × 11–14 μ. Nonparaphysate.

On pine panel, submerged 61 days below railroad bridge over Intracoastal Waterway, Beaufort, North Carolina, October 16, 1955, TYPE.

The principal diagnostic feature of this fungus is that of ascospore shape. The attenuated end-cell (FIG. 15) is not characteristic of any other species of *Sphaerulina*. *Sphaerulina pedicellata* is quite different from the following unnamed fungus in the same genus, and differs considerably in ascospore shape and size from *S. oraemaris*. It might be argued that inclusion of a fungus having longer ostioles than other representatives of the same genus distorts the generic limits. Ostiole length, however, varies considerably in sea-water agar cultures. Meyers has found this to be especially true with members of *Lulworthia* (= *Halophiobolus*).

SPHAERULINA sp. (FIGS. 4–6). Perithecia scattered, innate except for the upper portion of the short, papilliform ostioles, black above, light brown below, subglobose to lenticular, 301–426 μ in diameter, 154–216 μ high. Asci cylindrical or clavate, straight or curved, thin-walled, 8-spored, 125–150 × 10–15 μ, predominantly 130–142 × 10–12 μ. Ascospores fusoid or elliptical, hyaline, 5-septate, not constricted at septa; densely guttulate, partially to completely biseriate, 25–43 × 4–8 μ, predominantly 26–31 × 4–7 μ. Nonparaphysate.

Substratum: pine panels, submerged 36 days, off Carrot Island, Beaufort, North Carolina, July 29, 1955.

The characteristics agree in most respects with *Sphaerulina oraemaris* (Barghoorn & Linder, **1944**) from the California coast. The principal differences are those of ascospore septation and ascus size. The perithecia of this fungus are considerably larger than those of *S. oraemaris*.

There are apparently no species of *Sphaerulina* with strictly 5-septate spores. The nearest seems to be *S. vulpina* with 5–6-septate spores, or *S. intermixta* with 4–5-septate spores. The paucity of material precludes specific identification.

PHYSALOSPORA sp. (FIGS. 8, 14, 17). Perithecia scattered or gregarious, innate except for the short, papilliform ostioles, black, coriaceous, globose or subglobose, 326–590 μ in diameter, 216–432 μ high. Asci cylindrical or clavate, 8-spored, thin-walled when mature, 81–103 × 10–14 μ. Ascospores ovoid, hyaline, nonseptate, nonguttulate, surrounded by a hyaline sheath, uniseriate, 18–24 × 10–14 μ. Paraphyses simple or sparingly branched.

Substratum: birch veneer, submerged 3 months, Pivers Island, Beaufort, North Carolina, August 3, 1955.

The generic position of this fungus is tentative. It has the gelatinous-sheathed spores of *Sordaria* but the more pronounced ostioles of *Physalospora* (FIGS. 14, 17). The spore color is that of the latter genus. Since the material on which the description is based is sparse, specific identification must await more abundant collections.

PERITRICHOSPORA INTEGRA Linder, *In* Barghoorn & Linder, Farlowia **1**: 414. 1944. (FIGS. 22, 23). Collected on submerged pine panels and on driftwood. The ascospores are slightly larger (41–53 × 6–12 μ, inclusive of apical appendages) than those described by Linder. The spores are very characteristic, and cannot be confused with those of any other known marine fungus.

Höhnk (**1954**) described *Arenariomyces*, a genus with two species, *A. cinctus* and *A. trifurcatus*, neither of which was designated as the type. There is absolutely no doubt that *A. cinctus* is precisely the same as *P. integra*. The fact that *A. cinctus* developed perithecia on sand grains is a characteristic hardly worthy of according specific rank. *Arenariomyces cinctus* Höhnk is synonymous with *Peritrichospora integra* Linder, and the latter has priority.

The matter of *Arenariomyces trifurcatus*, however, poses a further problem. This species very closely resembles Meyer's (**1954**, fig. 2H) Form 7, and he concurs that the similarity is striking (communication). The matter is further complicated by the monotypic genus *Palomyces* (Höhnk, **1955**). The ascospores of *Palomyces quadri-remis* differ from those of Meyer's Form 7 only by the presence of 4 hyaline appendages on the end cells. Form 7 produces 3 such appendages. *Arenariomyces trifurcatus* and *Palomyces quadri-remis*, therefore, are much more alike in ascospore shape and appendages than are *A. trifurcatus* and *A. cinctus* (*Peritrichospora integra*). It is very doubtful that *A. trifurcatus* and *Palomyces quadri-remis* should be in separate genera despite their difference in habitat.

LULWORTHIA MEDUSA (E. & E.) Cribb & Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10): 80. 1955. (FIGS. 24–26, 30, 31). The species was collected on driftwood and on culms of *Spartina alterniflora*. On both substrates, the ascocarps are short-ostiolate and nearly completely innate. The hyaline ascospores are predominantly 360–405 × 4–4.5 μ.

Cribb and Cribb (**1955**) have shown Linder's genus *Halophiobolus* (Barghoorn & Linder, **1944**) to be antedated by Sutherland's genus *Lulworthia* (**1916**), and have combined all known species of *Halophio-*

bolus under the latter generic name. *Ophiobolus medusa* Ellis and Everhart (**1885**) and *Halophiobolus medusa* (E. & E.) Linder are synonyms of *L. medusa*. *Lulworthia longispora* (Cribb & Cribb, **1955**, p. 80) is probably only a variant of *L. medusa*.

LULWORTHIA SALINA (Linder) Cribb & Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10): 80. 1955. (FIGS. 27–29, 32). The characteristics of the two collections from pine panels (submerged 7 months) agree reasonably well with the fungus described by Linder, except for the greater size of ascospores, which are predominantly 205–215 × 3.2–4.8 μ. The perithecia are slightly larger (160–310 μ in diameter, 258–303 μ high), than described by Linder.

REMISPORA MARITIMA Linder, *In* Barghoorn & Linder, Farlowia **1**: 410. 1944. (FIGS. 36–38). A single collection from driftwood completely submerged at high tide. The ascospores, which are conspicuously constricted at the septum (FIG. 38), are predominantly 34–44 × 14–17 μ. The gelatinous appendages (FIG. 38), although usually paired, are occasionally 3–4 in number.

CERATOSPHAERIA sp. (FIGS. 33–35). Perithecia scattered, innate except for apex of the long, black, straight or curved ostioles; black, coriaceous, basal portion globose, 250–325 μ in diameter, 575–650 μ high. Asci cylindrical or clavate, thin-walled when mature, except for thickened apex; 8-spored, 155–190 × 14–22 μ. Ascospores clavate or cylindrical, hyaline, 2–3-septate, not constricted at septa; end cells with a small, hyaline cap; densely guttulate, uniseriate to biseriate, 41–50 × 5–9 μ, predominantly 43–46 × 6–9 μ. Paraphyses sparse or lacking; when present, short, simple.

Substratum: yellow poplar panel, submerged 3 months at nun buoy, Radio Island, Beaufort, North Carolina, September 16, 1955.

A very sparse collection. Inclusion of this fungus in *Ceratosphaeria* may well be questionable. The majority of the species in the genus produce superficial or partially innate perithecia. The marine fungus, however, consistently has perithecia which are innate except for the ostiolar apex (FIG. 33). The paraphyses are sparse or lacking. This characteristic, similarly, might justify excluding the fungus from *Ceratosphaeria*, since the known species are paraphysate. No species of *Ceratosphaeria* are described as having hyaline "caps" on the end-cells of the ascospores. The perithecia of *Metasphaeria australiensis* (Cribb & Cribb, **1955**) are very similar to those of this *Ceratosphaeria*. The spores of *M. australiensis* are constricted at the septa, however. Erection of a new genus is not warranted, although the characteristics of the fungus do not agree precisely with those of any of the known sphaeriaceous genera.

UNDETERMINED PLECTOMYCETE

A very sparse collection of a marine Ascomycete producing 4-spored asci in cleistothecia, was found on a pine board attached to a sunken piling off Shackleford Point, in open water. The characteristics are such that it is difficult to place the fungus in any of the previously described ascomycetous families with any degree of certainty. A description follows.

Ascocarps nonostiolate, globose, black, coriaceous, superficial, attached to substratum by slender, sparingly branched, dark brown hyphae; 56–104 μ, predominantly 70–88 μ in diameter. Asci globose-ellipsoid or broad-clavate, often irregular; thin-walled when spores are fully delimited, 4-spored, 24–30 × 15–19 μ. Ascospores hyaline, ellipsoid or botuliform, nonseptate, guttulate, 11–14 × 4–6 μ. Nonparaphysate. (FIGS. 42, 43).

Numerous small, nonostiolate fructifications were found scattered among the ascocarps in which asci were observed. There is little doubt that the small ascocarps were immature fructifications developed on the continually submerged substratum. The fungus did not grow on seawater agar or on various common mutrient agars such as malt and potato-dextrose. Neither ostioles nor ostiolar pores were in evidence on any of the ascocarps. The asci are probably not produced in locules, but this could not be determined definitely from the material at hand.

The affinities of the fungus are not determinable; most evidence points toward a relationship to the sooty molds, however. Assignment to a specific taxon will be dependent upon whether or not an ostiolar pore is produced, and the position and origin of the asci. It is entirely possible that mature fructifications were not collected.

SUMMARY

Six Deuteromycetes and nine Ascomycetes are reported from marine habitats. *Stemphylium maritimum*, *Massariella maritima*, and *Sphaerulina pedicellata* are new to science. Marine fungi reported but not identifiable because of paucity of material are tentatively recognized to be members of the genera *Hormiscium*, *Sphaerulina*, *Physalospora*, and *Ceratosphaeria*. *Arenariomyces cinctus* Höhnk is concluded to be synonymous with *Peritrichospora integra* Linder. A marine Ascomycete forming cleistothecia is described but not named.

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THE PLEUROTUS–HIRTUS–COMPLEX

ROLF SINGER

(WITH 3 FIGURES)

The genus *Pleurotus* in the narrower sense as I have defined it (**2**) is by no means a large one. It contains, however, a small number of complex groups which have been lumped together by the majority of the authors into some kind of "macrospecies" or rather accumulations of similar hereditary groups. One of these is the *Pleurotus-ostreatus*-complex; another is the *Pleurotus-hirtus*-complex with which the present paper is concerned.

In the wider sense, this complex of ill-defined forms is known as *Pleurotus hirtus* (Fr.) Sing. (or, incorrectly, as *Panus hirtus* Fr.) with a large number of synonyms. The types of some of these synonyms have been restudied by both R. W. G. Dennis and R. Singer (**1, 3, 5**) but when it comes to breaking down the linnaeon into smaller units it becomes increasingly difficult to tell with any degree of certainty which type belongs to which form. Consequently, in the following taxonomic study, we refrain from synonymizing all the species belonging in the complex, choosing merely one well documented binomial for each form. This may mean that the names applied may not stand up in view of a renewed revision of the pertinent type specimens since it is not fully demonstrable that in each case the name accepted by us is also the oldest specific epithet available. It is assumed, however, that the importance of the *Pleurotus-hirtus*-complex in the American tropics justifies a non-monographic treatment at this time inasmuch as this will make it possible to revise the types not treated here (or mentioned without definite determination) on the basis of the important diagnostical characters used to distinguish the constituent forms of the complex so far as they are known to the present author.

The descriptions and keys published here are partly based on data obtained during a recent collecting trip to the Departamento of Orán (Argentina, Province of Salta) where the subtropical rain forest known as Selva Tucumano-Boliviana becomes—geographically as well as "in facie" although not climatically—truly tropical and merges with the

Bolivian yungas which, on their part, connect the Selva Tucumano-Boliviana with the western extension of the Amazonas Basin. Naturally, the fungous flora becomes poorer as it approaches the southernmost portion of the Selva near Tucumán, and such elements as *Pleurotus hirtus* sensu lato (together with *Marasmius pallipes* Speg., tree ferns, *Ficus*, etc.) are still present in the rain forest of Orán, but wanting in the province of Tucumán. The *Pleuroti* and *Pani* of the former region are of some economic importance since they attack healthy trunks of lapacho (*Tabebuia* sp.), the main product of the northern lumbering industry, if cut during or just before the rainy season and left exposed to fungus attack in various railroad yards of the region before they are shipped south. I could observe several cases of originally healthy trunks which were severely attacked (and made practically worthless) by the rot caused by representatives of the *Pleurotus hirtus* complex, especially *P. calyx* (see below), by representatives of the *Pleurotus ostreatus* complex, especially the white, thin, tropical form with pale drab (almost white) spore print, and by *Panus rudis* Fr.

Observations in Orán and elsewhere in South America seem to indicate that externally and microscopically very similar forms hitherto determined as *Pleurotus hirtus* can be subdivided into four basic forms which we call *Pleurotus hirtus* sensu stricto, *P. calvescens* (Berk.) Sing., *P. concavus* (Berk.) Sing., and *P. calyx* (Speg.) Sing. The species described by Dennis (**1**) under the name of *Lentinus nigro-osseus* Pilát is probably a fifth representative of the same stirps.

PLEUROTUS HIRTUS (Fr.) Sing., Lilloa **22**: 271. [1949] 1951.
Panus hirtus (Fr.) Fr., Epicrisis 398. 1838.
Agaricus hirtus Fr., Linnaea **5**: 508. 1830.

Pileus white or sordid white, conspicuously velutinous to tomentose-rough all over and remaining so, almost circular, deeply infundibuliform, smooth, dry, with involute margin at first and remaining so in most specimens, thin, about 70–145 mm broad. Lamellae white or whitish, deeply descendent-decurrent, moderately broad, close, with entire, eventually sometimes somewhat lacerate edge, inserted, simple. Stipe white or sordid white, velutinous or tomentose-rough but not scaly, dry, 20–50 × 7–19 mm, central, or slightly to very strongly eccentric; no trace of a veil. Context fleshy-tough, relatively soft in age in wet weather, inodorous. Spores 6–8 × 2.7–3.8 μ, oblong to cylindrical, nor or scarcely curved, smooth, hyaline, inamyloid; basidia 29 × 3.7 μ, 4-spored; cystidia none; hymenophoral trama completely irregular, consisting of thick-walled, occasionally almost solid hyaline hyphae; sub-

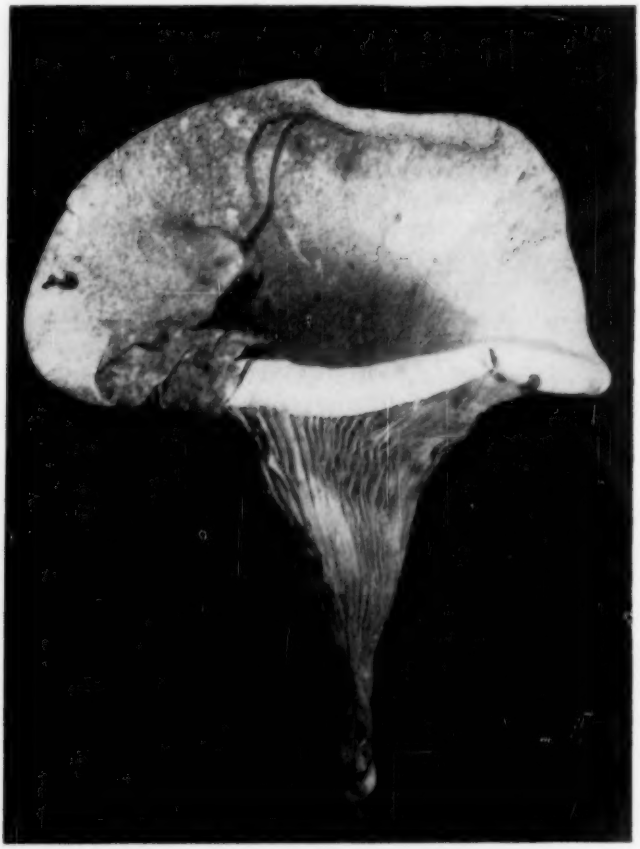

FIG. 1. *Pleurotus hirtus* (Fr.) Sing. Dried fruiting body, natural size, from collection T *2303*.

hymenium well developed, broad; all hyphae inamyloid, with clamp connections.

On dead wood of dicotyledoneous trees.

This species differs from the following ones in the scarcely glabrescent pileus, absence of a veil, and naked pileus and stipe, perhaps also in the somewhat larger, especially broader mature spores in an average. *Lentinus vellereus* and *Panus infundibulus* Berk. & Curt. differ only in distant lamellae.

Pleurotus calvescens (Berk.) comb. nov.

Lentinus calvescens Berk., Jour. Bot. & Kew Misc. **8**: 141. 1856.

Pileus white, glabrous, but at first slightly pubescent especially in the marginal regions and sometimes near the disc, dry, naked, smooth, with involute margin, later often irregularly lobed, convex, becoming somewhat depressed on disc and flattened otherwise, eventually infundibuliform, in dried material often buffish and slightly radially striate, 70–160 mm broad. Lamellae white, subdistant to close, deeply decur-

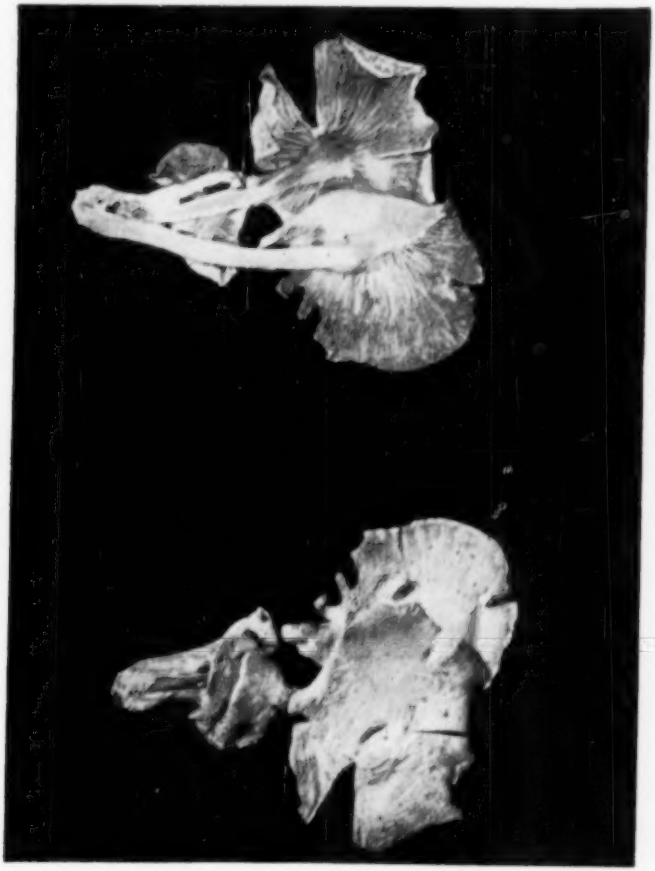

FIG. 2. *Pleurotus concavus* (Berk.) Sing. Dried carpophores, natural size, from collection Vargas 6199.

rent to a definite level, not anastomosing or forked at stipe, rather broad to broad, with entire edge, in dried material tending to be cream color. Stipe white, equal or slightly tapering upwards, glabrous, naked, smooth, only with the fibrils of the basal mycelium which is white, solid, subcentral, 10–25 × 4–11 mm. Context white, rather thick in the pileus, rather soft-elastic but not fleshy, inodorous. Spores 5.3–7.8 × 1.7–2.4 μ, cylindrical and often somewhat curved, smooth, hyaline, inamyloid; basidia 24 × 4.5 μ; cystidia none; cheilocystidia present but inconspicuous; hymenophoral trama consisting of thick-walled hyphae which run in all directions, irregular; subhymenium well differentiated; pubescence of young specimens consisting of hyphal ends which are cylindrical and about 2.8–3.5 μ broad, hyaline; all hyphae non-amyloid, with clamp connections.

Gregarious to cespitose-fasciulate on dead wood of dicotyledonous trees.

According to Dennis, the spore print is cream colored. Dennis identifies *Agaricus jacksonii* Berk. & Cooke, from Brazil, with this species. A very similar species is **Pleurotus concavus** (Berk.) comb. nov. (*Panus concavus* Berk.):

PLEUROTUS CONCAVUS (Berk.) Sing.

Panus concavus Berk., Ann. Mag. Nat. Hist. Ser. 2, **9**: 194. 1852.
Lentodiellum concavum (Berk.) Murr., Mycologia **7**: 216. 1915, vix sensu Murr., non sensu Dennis (**1, 6**).

Pileus white, glabrous, naked, smooth, in dried condition slightly radially striate, in mature specimens with straight margin, thin, convex with sharply depressed center, then flat with subacute umbilicus or deep depression in center, soon becoming all over deeply infundibuliform, 20–40 mm broad. Lamellae white, in dried condition somewhat buffish, simple, deeply decurrent, narrow, or almost so, not or scarcely anastomosing, inserted, at first arcuate, then descendant, close to crowded, with entire edge. Stipe white or whitish, glabrous, smooth, naked, without the slightest traces of a veil even in very young caps, equal, connate at base, 27–43 × 2–4 mm; basal mycelium scanty, thin-tomentose, white. Context white, elastic-tough. Spores 5.5–8.2 × 1.7–2.7 μ, cylindrical, some slightly curved, smooth, hyaline, inamyloid; basidia 15–24 × 4–4.8 μ, 4-spored; cystidia none; cheilocystidia inconspicuous; hymenophoral trama irregular, varying from entirely composed of thick-walled hyphae, intermixed thick- and thinned-walled hyphae; subhymenium forming a very distinct, rather broad layer; epicutis of pileus consisting of more or less repent hyphae, little differentiated; hyphal pegs not present in the hymenophore; all hyphae inamyloid and with clamp connections.

FIG. 3. *Pleurotus calyx* (Speg.) Sing. Dried carpophores, natural size, from collection T *2314*.

Cespitose to fasciculate on dead trunks (host not determined), fence rails, etc.

Pleurotus calyx (Speg.) Sing., comb. nov.
Agaricus calyx Speg., An. Soc. Cient. Arg. **16**: 243. 1883.
Clitocybe calyx (Speg.) Sacc., Sylloge **5**: 181. 1887.

Pileus white, fuscous to blackish fuliginous-scaly in the manner of some forms of *P. dryinus*, or *Panus tigrinus* in which, however, the

scales are fugacious and disappear in adult and old caps, finely white-pubescent in marginal region when young, later glabrescent in some specimens, convex with depressed center, eventually often truly infundibuliform, vaguely sulcate in marginal region when mature and especially when dry, 95–155 mm broad. Lamellae white, close to crowded, narrow or almost so (4 mm broad in large mature specimens), deeply decurrent, with entire edge, not forked at stipe, but often with low and scattered anastomoses on lamellar ground everywhere, tending to become pale cream buff in dried material; spore print pale ocher-cream. Stipe white, with transversal fuscous to blackish scales as in *Panus tigrinus*, these ending abruptly at the very apex of the stipe and thus with an annular zone, soon both annular zone and scales disappearing, the stipe remaining naked, glabrous, equal or slightly tapering upwards, solid, sometimes compressed, with white basal mycelium, in young specimens longer than the pileus diameter, in adult specimens usually short, 25–50 × 6–8 mm. Context white, mild, fleshy-tough, with a slight woody odor. Spores 6.5–8.7 × 1.7–2.8 μ, cylindrical, often curved, hyaline, smooth, inamyloid; basidia small; cystidia none; hymenophoral trama consisting of thick-walled (but hardly solid) hyphae, completely irregular; subhymenium well developed, forming a broad layer; all hyphae filamentous, inamyloid, with clamp connections.

On dead dicotyledonous trunks, e.g., *Tabebuia* sp., gregarious, sometimes in dense groups but not fasciculate, more rarely in small (2–4) fascicles, connate at base (FIG. 1).

This species, when seen fresh and young, has somewhat the appearance of *Pleurotus dryinus* (Pers. ex Fr.) Kummer, but differs in much smaller spores. When old, it resembles *P. hirtus* to such a degree that one is inclined to consider them a single species.

While this latter species is known only from Argentina and Paraguay, the others seem to have wide distribution in the American tropics. *P. hirtus* is known from Brazil and the northernmost forests of Argentina. *P. concavus* occurs all through the tropics from Santo Domingo to Peru, but has not been found in Argentina. *P. calvescens* was described from Brazil, but occurs undoubtedly in Cuba, and the Antilles and perhaps south to Argentina.

My material comes from:

1. *P. hirtus:* Brazil (type); Orán, prov. Salta; Argentina, coll. Singer T *2303*.

2. *P. calvescens:* Cuba, Soledad, Cienfuegos, coll. W. L. White *580*.

3. *P. concavus:* Santo Domingo (type); Jamaica, Balaclava, coll. Wight; Peru, Cadena, Cuzco, coll. C. Vargas C *6199*.

4. *P. calyx:* Paraguay, authentic material and type; Argentina, Pichanal, Dep. Orán, prov. Salta, coll. Singer T *2314*.

KEY TO THE SPECIES OF THE HIRTUS-COMPLEX

A. Carpophores initially with dark scales on pileus and stipe.....*P. calyx*
A. Carpophores naked.
  B. Pileus large (70 mm and larger) and stipe relatively short in mature specimens; surface of young pileus velutinous, hirsute, or tomentose.
    C. Surface of adult pileus conspicuously velutinous, hirsute, or tomentose; spores rarely curved and up to $8 \times 3.8(-4)\mu$.....*P. hirtus*
    C. Surface of pileus glabrescent, at least in mature specimens, in the zone between the margin and the center; spores narrow and tending to become curved.....*P. calvescens*
  B. Pileus smaller; stipe relatively not short in mature specimens; surface of young specimens glabrous or subglabrous excepting the basal mycelium.....*P. concavus*

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A NEW GENUS OF DOWNY MILDEW ON SORGHUM

P. N. Rao, M. A. Salam and M. J. Thirumalachar

(with 4 figures)

During the crop season of 1954, a downy mildew inciting spotting of leaves and grains of *Sorghum vulgare* was collected at the Agriculture Farm, Oosmania University, Hyderabad. The disease was unlike that of the downy mildew *Sclerospora sorghi* Weston & Uppal, which causes shredding of leaves, developing numerous oospores, and is widespread in the sorghum-growing areas of the states of Mysore, Bombay and Hyderabad. A detailed morphological study of the disease-inciting organism revealed that the fungus was an undescribed member of the Peronosporales, related in a general way to *Peronospora* and *Sclerospora*, but distinct from all the members so far described.

The disease has been noticed in restricted places in the field, and the extent of damage and its occurrence in other places are not yet known. Both leaves and the heads are affected by the fungus. On the heads, the mass of developing conidiophores and conidia appears as a greyish-white fuzz often involving the entire inflorescence. When carefully examined, the spots on the spikelets are numerous, minute, violet to yellowish-brown, occurring on both the glumes and grains. The stout, branched conidiophores emerge out of the host and bear numerous conidia, which give the downy appearance as in *Peronospora*. Following the maturation and dispersal of the conidia, the conidiophores do not collapse as in *Sclerospora*, but remain turgid and persistent as in *Peronospora*. Most of the infected grains get shrivelled or imperfectly developed, and hence in diseased heads there is poor filling of the grains (Fig. 2).

On the leaves, the spots at first appear as violet black specks covered with the downy growth of the condidiophores and conidia. Due to coalescence of the infection spots, large elongated patches are formed. There is no shredding of the leaves nor proliferation of the spikelets as in the case of other downy mildews of cereals and grasses.

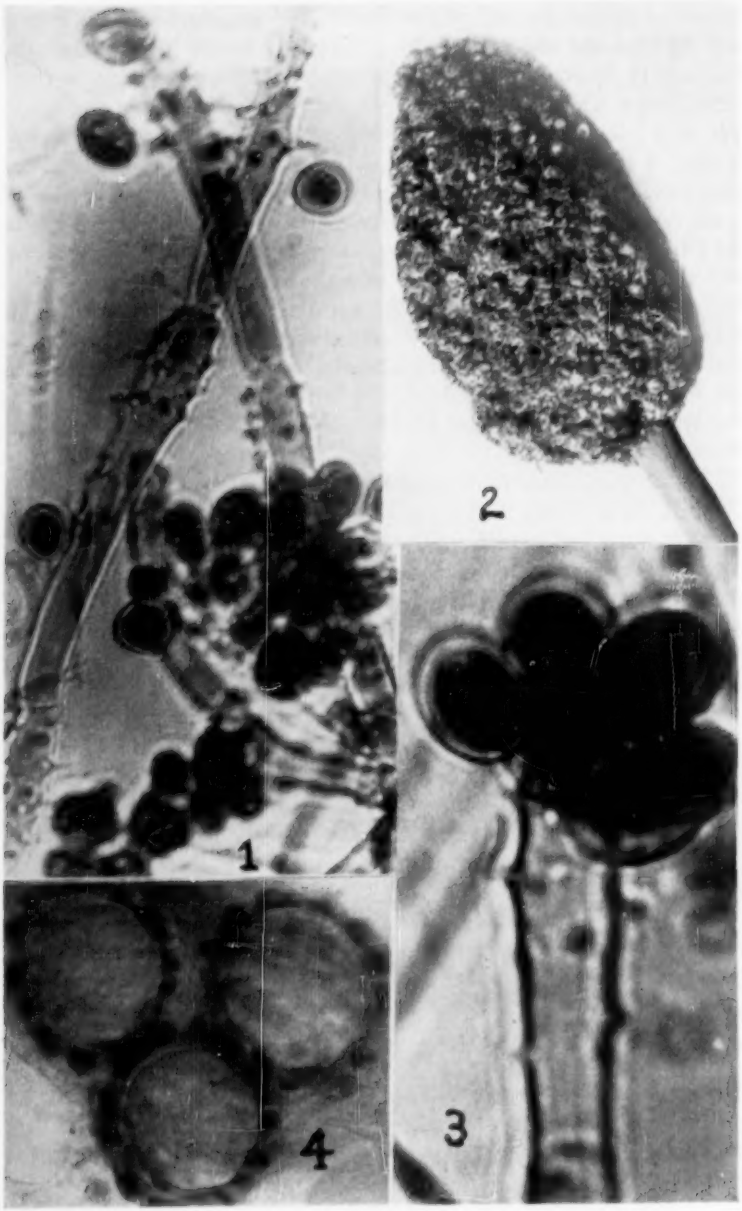
The mycelium is intercellular, hyaline, coenocytic, developing knob-like haustorial processes within the host cells. The conidiophores are distinct from the mycelium within the host and emerge vertically from

strands of hyphae through the stomata. The conidiophores are monopodially branched and septate, there being 4 to 6 cells in the main axis. Each cell of the conidiophore, except usually the lowermost one, is elongated, obconical, broader at the apex than at the base. At the apical half, each cell of the conidiophore develops numerous aculeate sterigmata bearing at their apices ovate to lemon-shaped conidia (FIG. 1). Consequently, the development of sterigmata and conidia is not restricted to the terminal portions of the conidiophores as in all other members of the Peronosporales, but is both terminal and intercalary. The terminal cells usually bear a single cluster of conidia (FIG. 3). When the conidia are dispersed, the long aculeate sterigmata remain persistent on the turgid conidiophores and appear as dentate structures. In their general appearance, the obconical cells bearing conidia have the aspect of the imperfect fungous genus *Physospora* Fries, but such resemblance is purely superficial, since the fungus under study is a member of the Peronosporales.

Mature conidia are ovate to lemon-shaped, hyaline, thin-walled, measuring 10–22 × 7–15 μ. The conidia germinate readily when placed on drops of water and incubated at room temperature (22–24° C). Germination takes place by the formation of a long germ tube with granular contents and is similar to that of species of *Peronospora*.

Oogonia and oospores are readily observed in sections of the infected leaf material. Oogonia are spherical, with the wall remaining persistent even after the oospores have matured! The undulate or papillate thickenings of the oogonial wall are characteristic of *Sclerospora* species and some species of *Albugo* on members of the Convolvulaceae in India. While stages of fertilization of the oogonia have not been observed, mature oospores are spherical, thick-walled, yellowish-brown, covered by the outer persistent wall of the oogonium, measuring 20–44 μ in diameter (FIG. 4). Germination of oospores has not been observed.

Taxonomy of the fungus. The occurrence of stout, branched conidiophores distinct from the hyphae within the host, developing a persistent down on the host and bearing oospores within the mesophyll tissue, indicates that the fungus under study is a member of the Peronosporales closely related to *Peronospora*. The conidia also germinate directly by germ-tubes as in *Peronospora*. The type of condiophore, consisting of obconical cells bearing conidia both in intercalary and terminal positions on aculeate sterigmata, is a character unknown in any member of the Peronosporales. A single terminal cell bearing a cluster of conidia at its apex is comparable to that of the condition in *Basidiophora*, but the



FIGS. 1–4.

presence of numerous such cells in a conidiophore with a monopodially branching system is unique. As the conidial or sporangial phase offers the distinguishing character in determining the genera of Peronosporales, it is proposed to present the fungus under study as a new genus of the Peronosporales, named in honor of Dr. James G. Dickson, University of Wisconsin, eminent investigator of diseases of cereal and forage crops of the world.

Dicksonomyces Thirumalachar, Rao & Salam gen. nov.

Mycelium parasiticum in plantis superioribus, hyalinum, coenocyticum, intercellulare. Status conidialis *Peronosporae* similis. Caespitulis effusis, lanoso-velutinis, griseo-albidis. Conidiophori e stomatibus exeuntes, ramosi, septati, quaque cellula obclavata, ad apicem sterigmata aculeata ferentes. Conidia hyalina, ovata vel ellipsoidea, germinantia per germinationis tubos. Status oogonialis similis *Sclerosporae*, parietes oogoniales crassi atque confluentes cum pariete oosporae.

Mycelium parasitic in higher plants, hyaline, coenocytic, intercellular. Conidial phase as in *Peronospora*, developing greyish-white downy growth. Conidiophores emerging out of the stomata, branched, septate, composed of cells which are obclavate, each bearing at its apex conspicuous aculeate sterigmata bearing conidia. Conidia hyaline, ovate to ellipsoid, germinating directly by germ tube. Oogonia and oospores as in *Sclerospora*, the wall of the oogonium being confluent with the wall of the oospore.

TYPE: *Dicksonomyces sorghi* Thirum., Rao & Salam on *Sorghum vulgare*.

Dicksonomyces sorghi Thirumalachar, Rao & Salam sp. nov.

Maculae minutae, atro-purpureae, in foliis, caulibus et partibus floralibus dispersae. Hyphae intercellulares. Conidiophororum caespituli effusi, lanoso-velutini, griseo-albidi, persistentes. Conidiophori e stomatibus emergentes, 300–500 μ alti, 4–6-ties ramosi, septati, quaque cellula obclavata, ad apicem sterigmata aculeata ferentes. Conidia hyalina, ovata vel ellipsoidea, 10–22 × 7–15 μ, germinantia per germinationis tubos. Oogonia subglobosa ad spherica, oosporae atro-brunneae, sitae in medio oogonii, sphericae, 20–44 μ in diam., quae intus continentur granulata atque nonnulas olei guttulas includentia, parietibus 2.5–3.5 μ crassis, hyalinis atque cum oogoni parietibus confluentibus.

Infection spots numerous, minute, purple-black, distributed on leaves, stem and heads. Hyphae intercellular. Conidia effuse, forming dense,

FIGS. 1–4. *Dicksonomyces sorghi*. 1. Portion of the conidiophore showing the obclavate cells with aculeate sterigmata and conidia, × 500. 2. Photograph of infected earhead, showing poor grain filling. 3. Enlarged view of the terminal conidiophore cell, showing cluster of conidia, × 1000. 4. Oospores, × 750.

greyish-white, downy growths which are persistent. Conidiophores emerging from stomata, 300–500 μ tall, 4–6-branched, septate, composed of cells which are obclavate, each of which develops at its apical end numerous aculeate sterigmata bearing conidia. Conidia hyaline, ovate to ellipsoid, 10–22 × 7–15 μ, germinating directly by germ tubes. Oogonia subglobose to spherical; oospores situated in the center of the oogonium, golden-brown, spherical, 20–44 μ in diameter, with granular contents and few droplets of oil; wall 2.5–3.5 μ thick, hyaline, confluent with the wall of the oogonium.

On leaves, stem and earheads of *Sorghum vulgare*, September 1954, Hyderabad, India, leg. P. N. Rao.

OOSMANIA UNIVERSITY, HYDERABAD
AND
PIMPRI, POONA, INDIA

THE GENUS RETINOCYCLUS[1]

J. Walton Groves and Doreen E. Wells[2]

(with 8 figures)

The genus *Retinocyclus* was erected by Fuckel (**1871**) based on *R. olivaceus* Fuckel, a Discomycete occurring on resin of *Larix*. The apothecia were described as olivaceous to black, subsessile, up to 2 mm broad, with a fleshy hymenium that was olivaceous when moist and black when dry. The asci were said to be 120 × 14 μ, multi-spored, and the spores perfectly globose, about 3 μ in diameter. Fuckel did not observe any conidial state.

Saccardo (**1889**) transferred Fuckel's species to the genus *Tromera*, which is now regarded as a synonym of *Biatorella*, a genus of lichens.

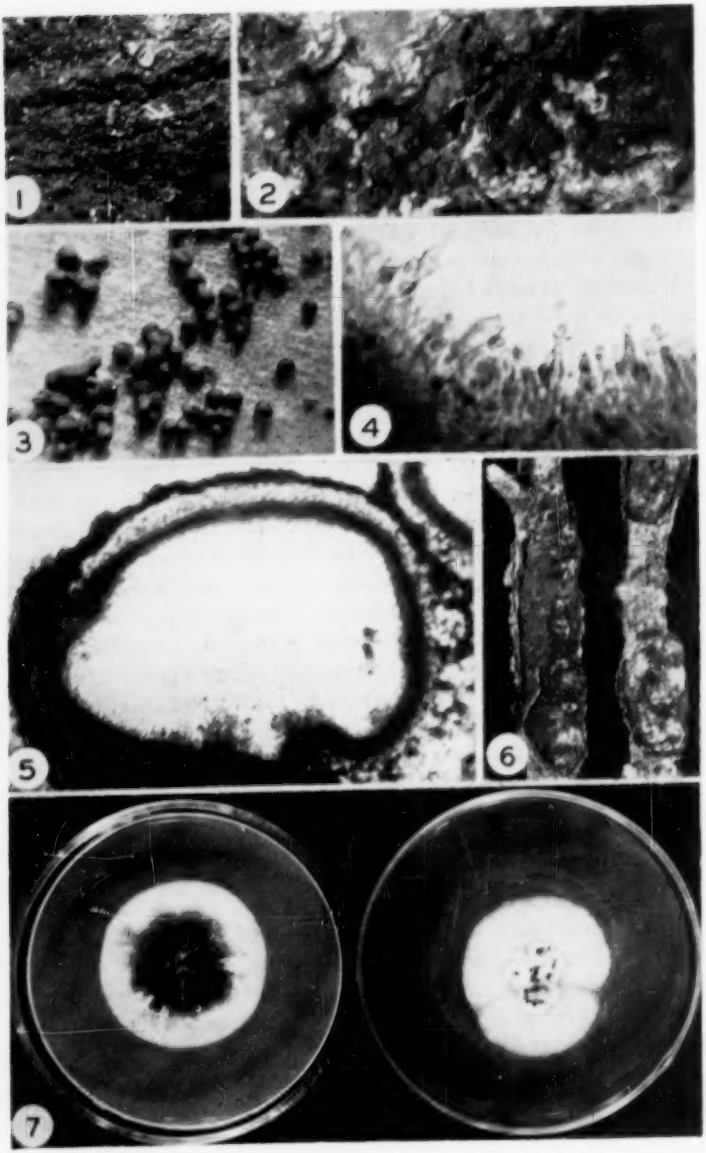
Rehm (**1889**) published a description based on the specimen in Fuckel's Fungi Rhenani *2475*. At that time he discarded Fuckel's genus and transferred the species to *Tympanis*, which it superficially resembles. He noted that the immature asci contained eight primary ascospores which were at first elliptic, becoming elongated and septate with up to seven transverse septa and occasionally muriform.

In a later account Rehm (**1912**) stated that he considered this species to be closely related to *Tympanis amphiboloides* Nyl., doubtless basing this opinion on the similarity in form of the primary ascospores. In this account Rehm reported the fungus as occurring on resin of *Pinus* as well as *Larix* and also as occurring on *Fagus*. The specimen on which this latter report was based, or at least a duplicate of it, was examined by the senior author in the herbarium of the Swiss Federal Institute of Technology, Zurich, and it proved to be a different fungus, *Corynella atrovirens* (Pers.) Boud.

We have not seen *R. olivaceus* in fresh condition but, through the kindness of Dr. J. R. Hansbrough, a specimen collected in Colorado by Henry Tiedemann on resin of *Picea engelmannii* was examined and it agreed morphologically with European specimens. This is apparently the first record of the fungus in North America. It has not been studied in culture and no conidial state has been described.

[1] Contribution No. 1540 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Principal Mycologist and Assistant Mycologist, respectively.



FIGS. 1–7.

In 1953 a *Tympanis*-like fungus was collected on resin of *Picea glauca* by R. J. Bourchier, Forest Biology Laboratory, Calgary, Alberta, and was sent to the senior author for identification. This fungus was similar in growth habit and consistency to *R. olivaceus* and also had multi-spored asci with globose, secondary ascospores. It was, however, clearly distinct from *R. olivaceus* by reason of the very much smaller asci. Cultures were obtained from ascospores and Mr. Bourchier was able to obtain further specimens which were also cultured. Subsequently, several isolates, received by the junior author among cultures sent to her for identification from the Forest Biology Laboratories at Saskatoon and Victoria, proved to be identical with the cultures isolated from ascospores of the Bourchier collection. Other collections of apothecia have also been made by members of the staff of the Forest Biology Division, extending the range to Manitoba, Saskatchewan, and British Columbia.

Among the species excluded from the genus *Tympanis* by Groves (**1952**) was *T. abietis* Crouan which was described as having globose secondary ascospores. The inadequate description by Crouan and Crouan (**1867**) did not give the size of the asci but LeGal (**1953**) examined the Crouan specimens and re-described and illustrated *T. abietis*, and her description and figures agree well with the Bourchier collections.

Accordingly some material of the Bourchier collection was sent to Madame LeGal with the request that she compare it with *Tympanis abietis* Crouan. She very kindly did so and reported (*in litt.*) that the two fungi appeared to be identical in every respect.

It is concluded that this fungus is *T. abietis* Crouan but because of the globose secondary ascospores, similar growth habit and consistency to *Retinocyclus olivaceus*, as well as its occurrence on resin, it should be transferred to *Retinocyclus*. The genus will then comprise two species that can be readily distinguished by the size of the asci.

Although the apothecia of *R. abietis* appear to be growing on resin and do not appear to be restricted as to host, the fact that they are associated with definite cankers (FIG. 6) raises the question as to whether or not there may be a causal relationship between this fungus and the canker. The fact that the fungus has been isolated on several occa-

FIGS. 1–7. *Retinocyclus*. 1. *R. olivaceus*. Apothecia of specimen in TRT ex herb. Barbey-Boissier *1456*, approx. × 4. 2. *Retinocyclus abietis*. Apothecia in DAOM, approx. × 4. 3. Conidial fruiting bodies of *R. abietis* on agar, × 20. 4. Section of conidial fruiting body of *R. abietis* showing conidia and phialides, × 900. 5. Section of conidal fruiting body of *R. abietis*, × 150. 6. Twigs of *Pseudotsuga taxifolia* with cankers on which apothecia of *R. abietis* were present, × 1.5. 7. Two isolates of *R. abietis* after about 8 weeks on malt extract agar, illustrating variation in amount of conidial fructification, × ½.

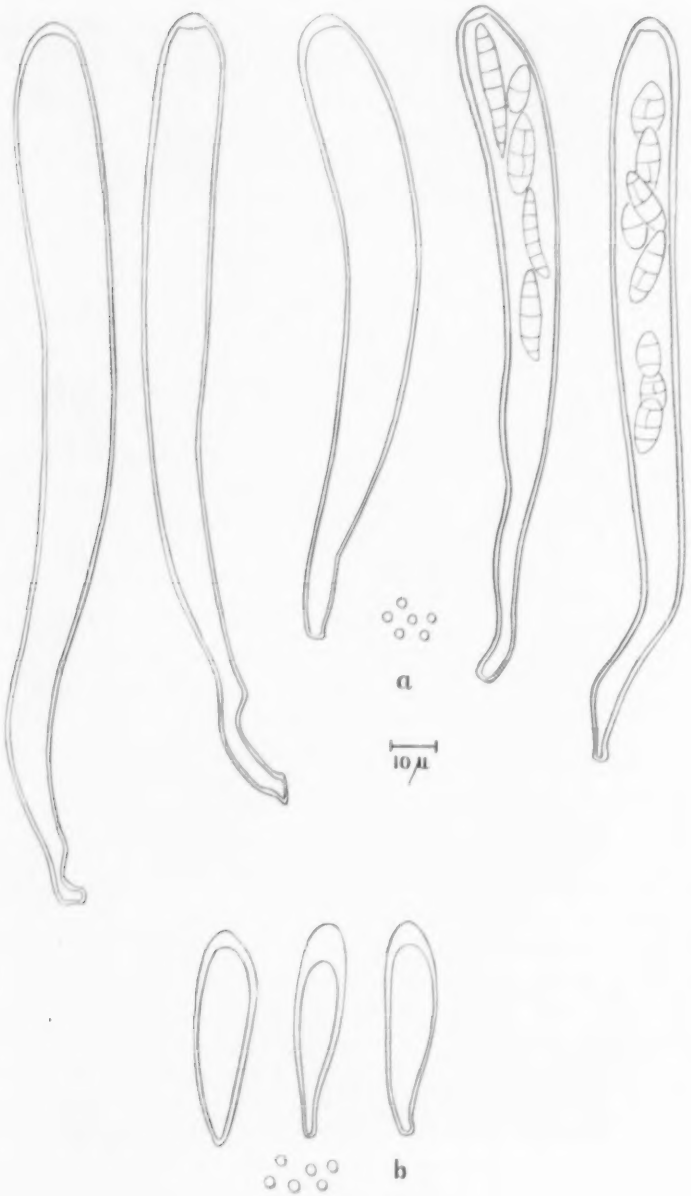


FIG. 8, a–b.

sions in different localities from cankered twigs is suggestive of such a relationship also. However, no investigation of the possible pathological significance of *R. abietis* has been undertaken.

The following descriptions have been based on the Barbey-Boissier and Tiedemann specimens for *R. olivaceus,* and the Canadian collections for *R. abietis.*

Retinocyclus olivaceus Fuckel, Jahrb. Nass. Ver. Naturk. **25–26**: 332. 1871. FIGS. 1, 8a

Tromera olivacea Sacc. Syll. Fung. **8**: 470. 1889.

Tympanis olivacea Rehm, Rab. Krypt.-Fl. I. **3**: 275. 1889.

Apothecia superficial, scattered, separate, circular or undulate, sessile, narrowed below, dull black, glabrous, 0.5–1 mm in diameter, 0.2–0.5 mm in height, hard, leathery to horny in consistency, becoming more fleshy-leathery when moist; hymenium concave to plane, dull black becoming olivaceous when moist, more fleshy than the excipulum; tissue of the hypothecium plectenchymatous, composed of hyaline to brownish, interwoven, ascending hyphae about 2–4 μ in diameter, with the walls somewhat thickened and gelatinized, curving toward the outside where the walls are thicker and darker, forming a rind-like excipulum; subhymenium not differentiated; asci cylindric to cylindric-clavate, tapering below to a slender stalk, at first 2–8-spored, finally multi-spored, (120–)150–190 × 16–19 μ; primary ascospores rather variable, ellipsoid to elongate-fusiform, up to 7-septate and sometimes muriform, uniseriate to partly biseriate, 11–28 × 3.5–7 μ; secondary ascospores globose, 1-celled, hyaline to greenish, 1.5–2.5 μ in diameter; paraphyses hyaline, filiform, septate, usually branched several times, especially near the tip, 2.0–2.5 μ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

On resin of *Larix, Pinus,* and *Picea.*

TYPE: Fuckel Fungi Rhenani *2475.*

Specimens examined: ex herb. Barbey-Boissier *1456* in TRT, FH, NYBG, BPI.

On *Picea engelmannii,* Upper Middle Park, Fraser Exp. For., Colo., Coll. H. Tiedemann, April 1950. No. *89950.*

Retinocyclus abietis (Crouan) comb. nov. FIGS. 2–7, 8b

Tympanis abietis Crouan, Fl. d. Finist. 43. 1867.

Apothecia erumpent-superficial, gregarious to scattered, mostly separate, occasionally subcespitose, black, glabrous, circular to undulate,

FIG. 8. a. Drawings of asci and primary and secondary ascospores of *R. olivaceus.* b. Drawings of asci and secondary ascospores of *R. abietis.*

sessile, slightly narrowed below, 0.5–1 mm in diameter, 0.3–0.5 mm in height, hard, horny in consistency, becoming softer and more fleshy-leathery to fleshy when moist; hymenium at first slightly concave, soon plane or sometimes convex, black or brownish when moist, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium compact, plectenchymatous, composed of closely interwoven, hyaline to brownish hyphae, 2.5–4 μ in diameter with the walls thickened and gelatinized, at the outside forming a thick, excipular layer of hyphae 2–3 μ in diameter with very greatly thickened and gelatinized walls, growing more or less parallel with each other and perpendicular to the outer surface, hyaline except the outer wall which is dark brown to black; subhymenium a dark brown zone of very closely interwoven, slender hyphae; asci clavate, short-stalked, thick-walled, especially at the apex, multi-spored, (43–)45–55(–60) × (11–)12–15(–16)μ; primary ascospores not seen; secondary ascospores hyaline, globose, 2–3 μ in diameter; paraphyses hyaline, filiform, septate, simple, 2–3 μ in diameter, the walls thickened and gelatinized so that they adhere together, and form a brownish gelatinous matrix at the tips, resulting in a pronounced epithecium.

On resin of *Picea glauca* (Moench) Voss and *Pseudotsuga taxifolia* (Poir.) Britton. It has also been isolated from *Pinus ponderosa* Dougl. and *Larix laricina* (DuRoi) Koch.

Pycnidia in culture (FIG. 3) abundant, separate to subcespitose, subglobose to conic, black, glabrous to minutely furfuraceous, 0.1–0.4 mm in diameter, fleshy-leathery, rather tough, containing a circular to ovoid or slightly lobed cavity (FIG. 5), splitting open at the top and the spores exuding in masses or cirrhi, the walls composed of a few layers of interwoven, very thick-walled hyphae, up to about 7 μ in diameter with the lumen 2–3 μ; conidiophores (FIG. 4) lining the cavity, and proliferated from branching hyaline cells, hyaline to fuscous, more or less flask-shaped with a flaring collar at the apex, 6–12 × 1.5–3.1 μ; conidia produced endogenously, fuscous, dark olive-brown to black in mass, subglobose, mostly narrowed at attachment end, 2–3.7 × 1.5–3.2 μ.

TYPE: in herb. Crouan.

Specimens examined: Alberta, DAOM *51072, 51073;* British Columbia, DAOM *51074, 51075, 51156;* Saskatchewan, DAOM *51091, 51155, 51157;* Manitoba, DAOM *51092, 51093, 51094.*

R. olivaceus has not been studied in culture but cultures were obtained from ascospores of *R. abietis* and were also isolated from twigs by members of the staff of the Division of Forest Biology and identified by comparison with the ascospore cultures.

On malt extract agar the fungus grows rather slowly (FIG. 7),

reaching a diameter of 2.5–3 cm in three weeks at room temperature. The colony is appressed-sodden, whitish to pallid, with a rather abrupt margin, producing pycnidia in more or less concentric zones. At first appearance it superficially resembles a *Tympanis* but is quite different in texture. Cultures of *Tympanis* species are soft and butyrous in consistency but cultures of *R. abietis* are tough and membranous.

The pycnidia were not observed in the material collected in nature but were produced abundantly in cultures (FIG. 3), both on agar and on sterilized twigs of various conifers. No conidial state has been described for *R. olivaceus,* but on examining sections of an apothecium from the Barbey-Boissier specimen one pycnidium was observed arising from the same stroma as the apothecium. This fruiting body was about 250 μ in diameter, with a wall about 40–50 μ thick composed of tissue similar to that of the apothecium, enclosing a lobed cavity lined with hyaline, filiform conidiophores, producing conidia that were similar in size and shape to the secondary ascospores. The section did not permit a satisfactory examination of the minute details of the conidiophores. However, the similarity between this single pycnidium and those produced in culture by *R. abietis* seems to provide additional evidence that these two species should be congeneric.

ACKNOWLEDGMENTS

The authors are indebted to Mr. R. J. Bourchier for material of *R. abietis* and to Dr. J. R. Hansbrough for material of *R. olivaceus.* Dr. D. P. Rogers and Mr. John A. Stevenson loaned specimens of *R. olivaceus.* We are especially grateful to Madame Marcelle LeGal for her kindness in comparing specimens with the type of *Tympanis abietis* Crouan.

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THE USTILAGINALES OF BIHAR. II.[1] FIVE UNDESCRIBED SPECIES

J. N. Mishra

(with 14 figures)

This contribution is a continuation of the previous studies on the smuts collected from different parts of Bihar. The types have been deposited in the Herb. C.M.I., Kew, England; Herb. Crypt. Ind. Orient., New Delhi; and in the Mycological Division, Plant Industry Station, Beltsville, Maryland, U.S.A.

Sphacelotheca ischaemi-rugosi sp. nov.

Sori in inflorescentia, glumis persistentibus inclusi, et reliquiis solidis textuum plantae hospitis columellam representantibus, e medio emergentibus et ad apicem furcatis. Peridium crassum, longitudinaliter ruptum ad monstrandos sporarum globulos agglutinatos nigrosque, ad monstrandas etiam cellularum sterilium catervas. Cellulae steriles peridii firmiter unitae interse, atque sporis multo minores. Sporae rubro-brunneae, rotundatae vel subglobosae, magnitudine regulares dimetientes 9.5–12.5 μ diam. (magnit. media 11.5 μ); exosporium tenue et aculeatum.

Sori (Fig. 1) in inflorescence, about 1.5 to 2 cm long, enclosed by persistent glumes and with stiff remains of host tissue representing columella, projecting from the center and forked at the tip. Peridium thick, rupturing longitudinally to expose the black agglutinated spore mass and groups of sterile cells interspersed into the sorus. Sterile cells of the peridium firmly united and much smaller than spores. Spores (Fig. 3) reddish-brown, round to subglobose, regular in size, measuring 9.5–12.5 μ with a mean of 11.5 μ in diameter; exospore thin and aculeate.

Hab. In inflorescence of *Ischaemum rugosum*, Netarhat, Bihar, India, alt. about 3000 ft., leg. J. N. Mishra. Type.

Ischaemum rugosum is a common weed of pasture land, where it is subject to heavy infection. *Sphacelotheca ischaemicola* Ling differs from the fungus under study in that the latter attacks the entire inflorescence and its spores are regularly globose and thin-walled with aculeate epispore.

[1] Part I in Mycologia **48**: 406–409. 1956.

Tilletia setariae-palmiflorae sp. nov.

Sori in ovariis, quae infecta remanent valde hypertrophiata, 3-5 mm. longi, 1–3 mm. diam., apicaliter sese aperientes ad liberandas sporas; ovarii infecti parietes crassi et coriacei. Sporae fusce rubro-brunneae, rotundae, 18.5–24.0 μ diam., magnit. media 20.8 μ; exosporium tenue, projectionibus alatis parvisque ornatum in vagina hyalina. Sporae steriles plures, hyalinae vel rubro-luteolae, plures appendicibus alatis parvisque ornatae, quae sese extendunt ad vaginam hyalinam, fere eiusdem magnitudinis ac sporae vel hisce minores.

Sori (FIG. 4) in some ovaries, infected ovaries much hypertrophied, 3–5 mm long and 1–3 mm in diameter, opening apically to release the spores, and walls of the infected ovary thick and leathery. Spores (FIG. 6) deep reddish-brown, round, 18.5–24 (average 20.8) μ in diameter; epispore thin, with small winged projections embedded in the hyaline sheath. Sterile spores numerous, hyaline to reddish-yellow, with small appendages on many of them continuous with the hyaline sheath, about the same size as or slightly smaller than spores.

Hab. In ovaries of *Setaria palmiflora*, Parasnath, Bihar, India, alt. about 3500 ft., leg. J. N. Mishra. TYPE.

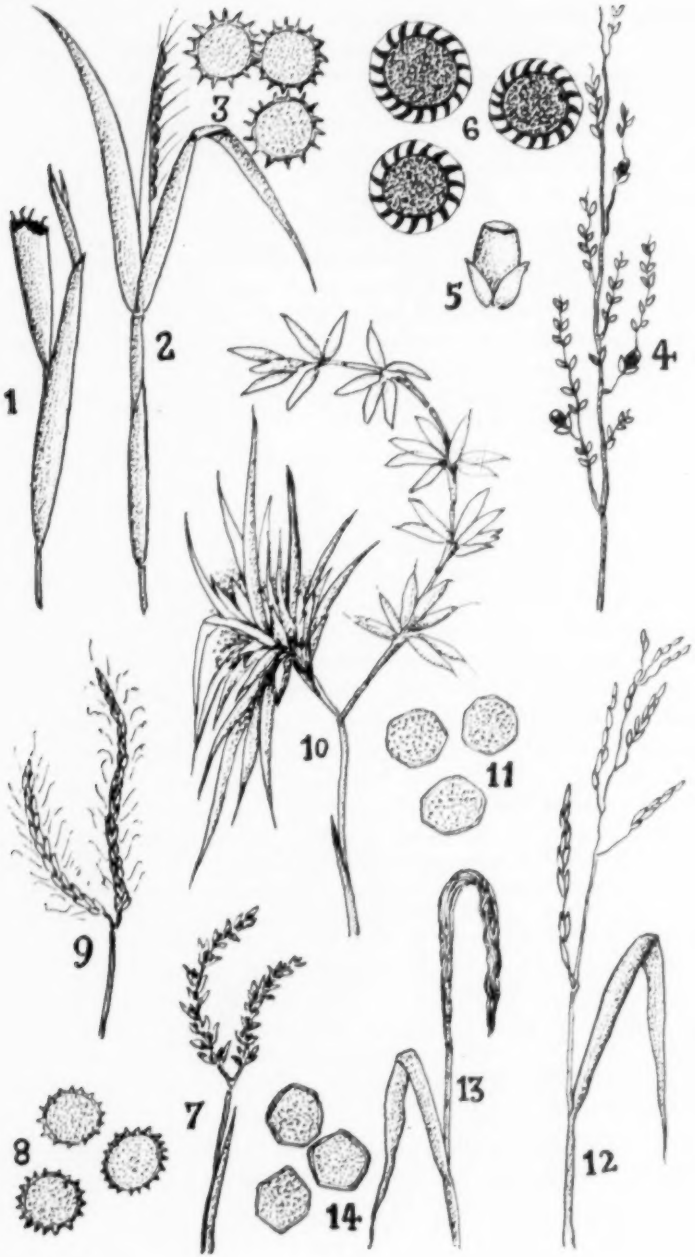
Setaria palmiflora, seen so far only in the hills of Parasnath, becomes heavily infected with the fungus under study. The infected ovaries of the panicle are conspicuous because they are much bigger than the normal ovaries, and they persist on the panicle long after the mature ovaries have been shed. The fungus differs from *Tilletia makutensis* Thirum. & Safee., whose sori are inconspicuous and opaque and whose spores have truncate scale-like projections. The sori of *T. setariae-palmiflorae*, which are much bigger than normal ovaries, have thick, bluish-green leathery walls, and the spores have small winged projections.

Ustilago sabouriana sp. nov.

Sori in ovariis, spiculas omnes plantae infectae destruentes, ca. 1 mm longi, inconspicui, glumis persistentibus inclusi, quae ad apices tenuiter aperiuntur ad monstrandos sporarum globulos nigros. Sporae rubro-luteae, rotundatae vel ovales, raro oblongae, magnit. 9.0–15.0 μ diam. (magnit. media 11.5 μ); exosporium rugosum vel sub lente immersa in oleum inconspicue echinulatum apparens.

Sori (FIG. 7) in ovaries destroying all spikelets of the infected plant, about 1 mm long, inconspicuous, enclosed by persistent glumes which open slightly at tips to expose the black spore mass. Spores (FIG. 8) reddish-yellow, round to oval, rarely oblong, measuring 9.0–15.0 (average 11.5) μ in diameter, exospore rugose to faintly echinulate under oil immersion lens.

Hab. In ovaries of *Bothriochloa pertusa* A. Cam., Netarhat, Bihar, India, alt. 3000 ft., leg. J. N. Mishra. TYPE.



FIGS. 1–3.

Bothriochloa pertusa, which is a common plant of the grassland, becomes infected both on the plateaus and in the plains. *U. sabouriana* comes very near *U. bothriochloae* Ling reported from China, but the infection due to the former is systemic and its spores are smaller, with rugose to faintly echinulate margins. *U. bothriochloae* infects only a few spikelets and its spores are echinulate.

Sorosporium apludae sp. nov.

Sori in spiculis aggregati in parvas magnasve proliferationes 3–4 cm longas, bracteis et glumis infectis evadentibus foliosis et valde elongatis. Sori persistentes, peridio brunneolo inclusi, monstrantes reliquias fibrosas textuum plantae hospitis, quae columellam representant, e medio emergentes. Sporarum globuli brunneoli, rotundati vel oblongi et elongati, nonnumquam triangulares, solidi vel ad maturitatem disintegrati, 66–180 μ longi, 50–100 μ lati, pluribus sporis constantes. Sporae rotundae vel polygonales, badiae, magnitudinis 7.5–12.0 μ diam. (magnit. media 9.6 μ); exosporium leve.

Sori (FIG. 10) in spikelets, grouped into small to large witch's brooms, 3–4 cm long, bracts and glumes of infected spikelets leafy and much elongated. Sori persistent, enclosed in brownish peridium, and with fibrous remains of host tissue, representing columella, sticking out from the center. Spore balls brownish, round to oblong and elongated, sometimes triangular, firm or slightly disintegrating at maturity, 66–180 μ long and 50–100 μ broad, composed of many spores. Spores (FIG. 11) round to polygonal, chocolate-brown, measuring 7.5 to 12.0 (average 9.6) μ in diameter; exospore smooth.

Hab. In spikelets of *Apluda varia*, Netarhat, Bihar, India, alt. 3000 ft., leg. J. N. Mishra. TYPE.

The fungus seldom infects the entire inflorescence. The infected spikelets are often found in small to large bunches which persist on the plant long after it is dead. The fungus has been collected only in the hills of Netarhat and Parasnath between altitudes of 2000 to 3000 ft. No *Sorosporium* appears to have been described on *Apluda*.

FIGS. 1–3. *Sphacelotheca ischaemi-rugosi*. 1. Infected plant of *Ischaemum rugosum*, × 1.5. 2. Healthy plant of same, × 0.5. 3. Spores of *S. ischaemi-rugosi*, × 1000. FIGS. 4–6. *Tilletia setariae-palmiflorae*. 4. Infected panicle of *Setaria palmiflora*, × 0.3. 5. Infected ovary of same, × 4. 6. Spores of *T. setariae-palmiflorae*, × 600. FIGS. 7–9. *Ustilago sabouriana*. 7. Infected inflorescence of *Bothriochloa pertusa*, × 2. 8. Spores of *U. sabouriana*, × 700. 9. Healthy inflorescence of *B. pertusa*, × 2. FIGS. 10–11. *Sorosporium apludae*. 10. Infected and healthy parts of inflorescence of *Apluda varia*, × 1. 11. Spores of *S. apludae*, × 800. FIGS. 12–14. *Sorosporium leersiae*. 12. Healthy shoot of *Leersia hexandra*, × 0.3. 13. Smutted shoot of *L. hexandra*, × 0.2. 14. Spores of *S. leersiae*, × 1300.

Sorosporium leersiae sp. nov.

Sori in inflorescentia, eam penitus destruentes, fasciis longis fibrosisque textuum plantae infectae columellam repraesentantibus ornati, quae emergit ex vagina foliorum, primo operti peridio delicatulo, quod in frustula rumpitur ad liberandos nigros sporarum globulos. Cellulae steriles peridii parvae, earumque catervae interspersae soro. Sporarum globuli sat firmi, rotundi vel ovati, nonnumquam polygonales, magnitudine regulari, 5.2–7.5 μ diam. (magnit. media 6.2 μ); exosporium leve et moderate crassum.

Sori (FIG. 13) in inflorescence, completely destroying it, with bunches of long fibrous remains of host tissue representing columella sticking out of leaf sheath, covered at first with a delicate peridium which flakes away early to release the black spore mass. The sterile cells of the peridium small and groups of them interspersed into the sorus. Spore balls rather firm, round to oval and sometimes triangular to elongate, light reddish brown to deep brown in color, measuring 40–90 μ × 25–60 μ. Spores (FIG. 14) reddish-yellow, round to oval, and occasionally polygonal, regular in size, measuring 5.2 to 7.5 (average 6.2) μ in diameter; exospore smooth and moderately thick.

Hab. In inflorescence of *Leersia hexandra* Sw., Ranchi farm, Bihar, India, leg. J. N. Mishra. TYPE.

Leersia hexandra is a common plant in the lowland. No *Sorosporium* appears to have been described on *Leersia* so far.

The author is thankful to Dr. M. B. Ellis, of the Commonwealth Mycological Institute, Kew, for his kind help and advice, and to the Rev. Father Dr. Santapau, of the Botanical Survey of India, for the Latin diagnosis.

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NOTES AND BRIEF ARTICLES

A NEW SPECIES OF PICOA

Picoa pachyascus sp. nov.

Ascosomata 3–5 cm lata, regularia. Peridium areolato-verrucosum, verrucis fuscis. Gleba pallida, dein sordide purpureo-fusca, venis inconspicuis. Asci pyriformes, 80–100 μ lati, parietibus crassis. Sporae fuscae, globulares, 36–43 μ latae.

TYPUS sub Rhododendro prope oppidum Highlands Carolinae Septentrionalis Americae Borealis mense Sept. 1947 a M. Lange lectus, in Museo Botanico Hauniensi depositus.

Fruit body 3–5 cm broad, somewhat lobed or almost regularly globose, with small mycelial tuft at base; peridium areolate-warty from very inconspicuous warts, more distinctly warty when dried, warts tawny,

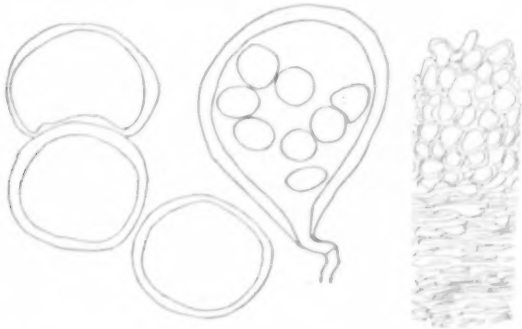


FIG. 1. *Picoa pachyascus*. 3 spores, × 500; ascus, × 250; drawn by aid of camera lucida. Section of peridium (semischematic).

pallid and slightly scurfy-hairy between warts; gleba pallid or almost white when immature, dark brown (bister) on mature, dried specimens, very indistinctly marbled from inconspicuous narrow veins; without sterile base, brittle like cheese. Smell and taste faint. Peridium 500–700 μ thick, including low warts about 100–200 μ high, warts and narrow outer part made up of pseudoparenchyma, the cells 15–50 μ broad, thick-walled, brownish in outer layers, a few prolonged into short, blunt hairs, inner main part of peridium of hyphal elements 6–8 μ broad or inflated and broader, subhyaline, continuous with gleba of same structure, hyphae septate and branched; asci evenly distributed over gleba, sub-

globose-piriform, 80–100 μ broad, wall very thick in young asci, and still about 10 μ thick when spores have developed to fill out the whole ascus, hyaline, ascus plasma granular, subhyaline, 8-spored, almost all asci distinctly stipitate; spores globose, thick-walled, long remaining hyaline, then brown, smooth, 36–43 μ broad, wall about 3 μ thick.

This species was found at Highland, N. C., during the 1947 annual foray of the Mycological Society of America, among roots of *Rhododendron*, in a thicket at the edge of a creek. There were five half-exposed fruit-bodies in the cluster. It differs from other American species of *Picoa* in its globose spores, but seems close to *P. lefebvrei* (Pat.) Maire, described from North Africa. However, the present species has the peridium less hairy and has considerably larger spores, and the very conspicuous thick-walled asci are not mentioned in the descriptions of *P. lefebvrei*. *Picoa juniperi* has more distinct venation and the peridium is more warty. It differs from the genus *Carbomyces* Gilkey in the areolate-warty peridium and the much larger spores.—MORTEN LANGE, Botanical Laboratory, University of Copenhagen.

THE NAMES DACRYMYCES STILLATUS AND DACRYMYCES ABIETINUS

In 1816 Nees (Syst. Pilze Schw. 89) set up the genus *Dacryomyces* with one species, *Dacryomyces stillatus*. The description merely states that the fructification is lobed, yellow to orange in color, deliquesces in old age and occurs on fir wood; however, the illustration almost certainly represents the imperfect stage of *Dacrymyces deliquescens* (Mérat) Duby.

Persoon's descriptions in the Synopsis Methodica Fungorum of 1801 and the Mycologica Europaea of 1822 are so lacking in specific detail that it is impossible to be certain of the species he described. It seems probable that his *Tremella abietina*, *Peziza abietina* and *Tremella deliquescens* were all what is now called *Dacrymyces deliquescens*.

Fries (Syst. Myc. **2**: 230. 1823) applied the name *Dacrymyces stillatus* Nees to the oidial stage of *Dacrymyces deliquescens*. In discussing this genus Fries changed the name from *Dacryomyces* to *Dacrymyces*, apparently deliberately. Both spellings appear with about equal frequency in the current literature. Since Fries's work is the starting point for the nomenclature of these fungi, and *Dacrymyces* is merely an orthographic variant of *Dacryomyces*, it seems proper to use the spelling adopted by Fries. Later (Hymen. Eur. 698. 1874), Fries listed the perfect stage of *Dacrymyces deliquescens*, based primarily on Duby's

treatment (Bot. Gall. 729. 1830), and cited *Tremella deliquescens* Bull. (Champ. France 219. 1791), and *Tremella lacrymalis* Pers., the latter probably a different species, as synonyms; no mention is made of the imperfect stage. At the same time *Dacrymyces stillatus* Nees was listed as possessing multiseptate spores and *Tremella abietina* Pers. (Syn. Meth. Fung. 627. 1801), was cited as a synonym. It is obvious that Fries was confused about these fungi for he applied the same name to different species with no explanation of the difference between his treatment of 1823 and that of 1874.

Tulasne (Ann. Sci. Nat. Bot. III. **19**: 219. 1853) used the name *Dacrymyces stillatus* in still another sense. It is clear from the description that his species was either a *Ditiola* or a *Guepiniopsis*. Tulasne noted that *Dacrymyces stillatus* Nees is the imperfect stage of *Dacrymyces deliquescens*. Later Bourdot and Galzin (Hymén. France 68. 1928) applied the name *Dacryomyces stillatus* Nees to the species Tulasne described. They failed to realize that *Dacrymyces stillatus* Tulasne is not a synonym of *Dacromyces stillatus* Nees, as Tulasne himself had pointed out.

Schröeter (Krypt.-Fl. Schles. **3**: 400. 1888), under the name *Dacryomyces abietinus* Pers., gave the first adequate description of the species *Dacrymyces stillatus* Nees sensu Fries 1874. He considered *Dacrymyces stillatus* Nees sensu Fries 1823 and *Tremella abietina* Pers. 1801 to be synonyms of his species. In this concept he was followed by Saccardo (Syll. Fung. **7**: 798. 1888); Brefeld (Unters. **7**: 155. 1888); Coker (Jour. Mitchell Soc. **35**: 161. 1920); Bresadola (Icon. Myc. Pl. 1126. 1932); Brasfield (Amer. Midl. Nat. **20**: 216. 1938, but open to question); and Martin (Univ. Iowa Stud. Nat. Hist. **19**(3): 30. 1952).

Neuhoff (Arkiv. för Bot. **28** A(1): 38. 1936) departed from the entrenched idea of *Dacrymyces stillatus* Nees ex Fries 1823 established by Schröeter. He stated that *Dacrymyces stillatus* Fries 1823 is the imperfect stage of *Dacrymyces deliquescens* and that *Tremella abietina* Pers. is a synonym.

As a result of this survey of the literature it seems clear that the name *Dacrymyces stillatus* has been applied to three different species: the imperfect stage of *Dacrymyces deliquescens* (Mérat) Duby, *Dacrymyces abietinus* Schröet. and probably *Guepiniopsis chrysocomus* (Fries) Brasf. Since so much confusion centers around this name it is suggested that it be considered a *nomen ambiguum* and rejected, at least until the relevant type material, if it still exists, can be examined.

Persoon's description of *Tremella abietina* is so scanty that it is suggested reference to this species be omitted.

The following synonymy is proposed for the two species which have been involved in this tangle of names. The names of historical interest are indicated by square brackets.

DACRYMYCES ABIETINUS Schröet. Krypt.-Fl. Schles. **3**(1): 400. 1888.
Dacrymyces stillatus Fries, Hymen. Europ. 699. 1874 (not *Dacrymyces stillatus* Fries 1823; not *Dacrymyces stillatus* Tul. 1853).

Dacrymyces deliquescens (Mérat) Duby, Bot. Gall. 729. 1830.
[*Tremella deliquescens* Bull. Champ. France 219. 1791.]
[*Tremella abietina* Pers. Syn. Meth. Fung. 627. 1801.]
[*Dacryomyces stillatus* Nees, Syst. Pilze Schw. 89. 1816 stat. imp.]
[*Peziza abietina* Pers. Myc. Eur. **1**: 104. 1822 stat. imp.]
Dacrymyces stillatus Fries, Syst. Myc. **2**: 230. 1823. stat. imp.

This work was done under the supervision of Professor G. W. Martin, Botany Department, State University of Iowa, Iowa City, Iowa.—
LORENE L. KENNEDY, BOTANY DEPARTMENT, UNIVERSITY OF ALBERTA, EDMONTON, ALBERTA.

NOTE CONCERNING CERCOSPORA ABELIAE

Recently the writer [1] published on a leaf spot of *Abelia grandiflora* and described the causal organism as a new species of *Cercospora* under the name *C. abeliae*. The disease was first noted in 1951 and the paper submitted for publication in August, 1955.

A search was made through the literature at the time the paper was being prepared for publication, and no description of a *Cercospora* on *Abelia* was found. However, after the publication of the paper, Dr. Charles Chupp kindly called my attention to a paper published by Katsuki [2] in December, 1955 in which *C. abeliae* was described as a new species on *Abelia sinensis*.

Katsuki's description and measurements fit closely those of mine. There are some differences, but these are probably not important when host and environmental differences are taken into consideration. The two most important differences are: (1) Katsuki states that the fruiting

[1] Plakidas, A. G. 1956. *Cercospora* leaf spot of *Abelia*. Mycologia **48**: 382–385.

[2] Katsuki, Shigetaka. 1955. New or noteworthy *Cercosporae* from Japan. III. 1. *Cercospora abeliae* Katsuki sp. nov. Ann. Phytopath. Soc. Japan **20**: 71–72.

bodies of his fungus are amphigenous; in the Louisiana fungus they are strictly hypophyllous and stomatal. (2) Katsuki describes conidiophores as continuous and simple; in the Louisiana fungus both branching and septation of the conidiophores were noted.

Notwithstanding these differences, it is accepted, at least tentatively until specimens have been compared, that the *Cercospora* described by the writer is the same as that described by Katsuki, and, of course, Katsuki's designation has priority. A. G. PLAKIDAS, Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana.

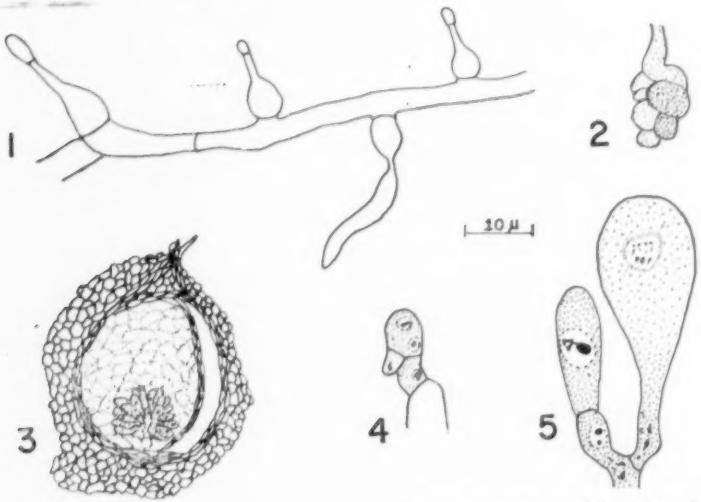
ON MELANOSPORA EPISPHAERIA

Martin (Mycol. **47**: 606–608) recently reported and illustrated a fungus isolated from leaf litter collected at Box Hill, Surrey, England, which he assigned to *Sphaeroderma episphaeria* (Phill. & Plowr.) Sacc. The present note presents some additional cultural observations of this fungus, as well as some discussion of its morphology and cytology.

Single spore cultures were obtained by diluting a spore suspension, and streaking the spores on a malt agar plate. In about two days, germinating ascospores were found by examining the plates with the low power lens of the microscope. A small block of agar containing a single germinated spore was transferred to a sterile plate of malt agar, and the mycelium allowed to develop. Each agar block was carefully examined to ascertain that it contained only one spore. Three such cultures were prepared. In about three weeks time, perithecia were present in all of the cultures. These matured and produced typical spores. The fungus thus appears to be homothallic.

In an attempt to determine the mode of perithecial development, the fungus was grown in Shoemaker slide cultures. Weak potato dextrose agar was used as the culture medium. Perithecial primordia appeared in about five days. The primordium is first evident as a coiled and somewhat inflated hyphal tip, usually appearing as a hypha recoiling upon itself, which is soon surrounded by a layer of pseudoparenchymatous cells (FIG. 2). Often, in young cultures, phialide-like structures are found in association with perithecial primordia. These are extremely variable in form and size, but typically are distinctly flask-shaped and narrow to a distinct neck. Occasionally 2-beaked phialides have been observed. No conidiophores are produced, and the phialides are scattered irregularly on the hypha which bears them (FIG. 1). They are about 7–14 × 4–6 μ. The conidia are globose to ovoid, 3–6 × 3 μ.

Sections were prepared by killing and fixing perithecia on blocks of agar in FPA, embedding them in paraffin, and sectioning at 6 μ. The sections were stained in iron alum-hematoxylin and counter-stained with orange G. Examination of these sections indicates that the ascogenous hyphae arise in a cluster at the base of the perithecium, the cluster then spreading in more or less fan-like fashion as the asci are formed (FIG. 3). The ascus appears to originate through the formation of croziers (FIG.



FIGS. 1–5. *Melanospora episphaeria*. 1. Phialides and conidia, viewed in fresh mount. The mycelium has proliferated through one of the phialides. 2. Perithecial primordia, seen in wet mount stained with phloxine. 3. Section through a perithecium, × 75. 4. Crozier, seen in section of a perithecium stained with iron alum-hematoxylin. 5. Immature asci, the smaller containing what is probably the fusion nucleus. The larger ascus contains what appears to be a telophase division figure.

Magnification of Figs. 1, 2, 4, 5 indicated by scale.

4), and contains 8 spores at maturity. The asci deliquesce early, and intact asci containing mature spores were found only infrequently in the sections. Immature asci, containing one to several nuclei, were present in several of the perithecia sectioned.

The genus *Sphaeroderma* was erected by Fuckel in 1875 and was based on *S. theleboloides*. This species was transferred to *Melanospora* by Winter (in Rabenh. Krypt. Fl. 2: 94). Petch (Jour. Bot. **74**: 191–193) considered *Sphaeroderma* to be a *Melanospora* without a beak.

Doguet (Le Botaniste **39**: 1–313) has made an extensive study of the genus *Melanospora* and follows Winter in treating *S. theleboloides* as a *Melanospora*. If this point of view is accepted, the genus *Sphaeroderma* is superfluous. Doguet recognizes Phillips and Plowright's fungus as correctly placed in *Melanospora* and with this I agree. The species is, therefore, to be called *Melanospora episphaeria* Phill. & Plow.

This work was done in the Mycological Laboratory of the State University of Iowa under the supervision of Professor G. W. Martin.—ROGER D. GOOS, Dept. of Botany, State University of Iowa, Iowa City, Iowa.

RESPIRATION OF AMINO ACIDS BY STREPTOMYCES NITRIFICANS [1]

There have been relatively few reports on the nitrogen metabolism of actinomycetes, as Gottlieb and Ciferri [2] have pointed out in a recent issue of MYCOLOGIA. Information on the ability of *Streptomyces nitrificans* to oxide amino acids contributes, therefore, to knowledge about a somewhat restricted area. *S. nitrificans* is unique in its ability to grow on urethan (*i.e.*, ethyl carbamate) as the sole source of nitrogen, carbon, and energy.[3] This organism is also a heterotrophic nitrifier which produces nitrite from ammonia, urea, guanidine, amino acids, purines, pyrimidines, carbamates, and other nitrogenous substances during growth and in respirometric studies.[4, 5, 6]

Urethan is an enzyme poison and mutagenic agent.[3] On this account, it was assumed that the pattern of substrate utilization by *S. nitrificans* might be significantly different when the organism was cul-

[1] This investigation was supported in part by a research grant (C-2120) from The National Cancer Institute of the National Institutes of Health, United States Public Health Service.

[2] Gottlieb, D. and O. Ciferri. Deamination and degradation of amino acids by streptomycetes. Mycologia **48**: 253–263. 1956.

[3] Schatz, A., H. D. Isenberg, A. A. Angrist and V. Schatz. Microbial metabolism of carbamates. I. Isolation of *Streptomyces nitrificans* n. sp., and other organisms which grow on urethan. Jour. Bact. **68**: 1–4. 1954.

[4] Isenberg, H. D., G. S. Trelawny and A. Schatz. The microbiological metabolism of urethan and other carbamates. Proc. VI[th] International Congress of Microbiology **1**: 143. 1954. Rome.

[5] Isenberg, H. D., A Schatz, A. A. Angrist, V. Schatz and G. S. Trelawny. Microbial metabolism of carbamates. II. Nitrification of urethan by *Streptomyces nitrificans*. Jour. Bact. **68**: 5–10. 1954.

[6] Schatz, A. and R. R. Mohan. Oxidation of ammonia and urea (heterotrophic nitrification) by *Streptomyces nitrificans*. Jour. Cell. Comp. Physiol. **45**: 331–342. 1955.

tured in a mineral solution supplemented with (a) urethan alone as compared with (b) peptone, glycerol, and yeast extract as more "normal" substrates.[7] To determine whether any such variation in amino acid metabolism occurred, Warburg experiments were carried out with cell material harvested from these two media and prepared in the manner reported elsewhere.[7]

The results (TABLE I) show that both cell lots respired glutamate most rapidly. The next highest rate of oxidation was observed with aspartate. Glycine, alanine, and serine were utilized more slowly,

TABLE I

RESPIRATION OF AMINO ACIDS BY S. NITRIFICANS

	Mycelium grown on	
	Urethan 0.2%	Peptone 0.5%, glycerol 0.5%, and yeast extract 0.2%
mg cell nitrogen/vessel →	1.4	1.8
Experiment, minutes →	90	130
Substrate, 10.0 μM per vessel	$Q_{O_2}(N)$ = μl O_2 consumed per mg of cell nitrogen per hour	
DL-Glycine	7	11
DL-α-Alanine	4	22
DL-Serine	3	18
DL-Aspartate	8	25
NaH glutamate	17	59
DL-Arginine	<1	1
None (autorespiration)	14	85

Warburg conditions were the same as previously employed.[7] $Q_{O_2}(N)$ values have been corrected by subtracting the respective autorespiration data.

while arginine was not handled at an appreciable rate. For these amino acids, lower $Q_{O_2}(N)$ values were obtained with urethan-grown mycelium, as had been observed in corresponding experiments on the oxidation of lactate and alcohols.[7] The relatively low rate of utilization of alanine was not appreciably increased by providing α-ketoglutarate, since *S. nitrificans* apparently lacks a transaminase for this system.[8]

[7] Mohan, R. R., G. S. Trelawny and A. Schatz. Microbial metabolism of carbamates. III. Preparation and respiratory activity of nonproliferating cells of *Streptomyces nitrificans*. Jour. Bact. **69**: 387–392. 1955.

[8] Schatz, A., R. R. Mohan and G. S. Trelawny. Oxidation of tricarboxylic acid cycle intermediates by *Streptomyces nitrificans*. Antonie van Leeuwenhoek **3**: 225–238. 1955.

In other studies, to be published later, urethan-grown *S. nitrificans* failed to respire carbamylglycine, carbamylalanine, carbamylglutamate, and carbamylphosphate. DL-Ornithine, DL-citrilline, and acetyl-L-glutamate were also not oxidized. It is, therefore, doubtful whether the urea cycle is operative in *S. nitrificans*, at least when this actinomycete develops on urethan as the sole nitrogen, carbon, and energy substrate. —A. SCHATZ, G. S. TRELAWNY, V. SCHATZ AND R. R. MOHAN, National Agricultural College, Doylestown, Bucks County, Penn.

MYCOLOGICAL PROGRAM AT NEW YORK MEETING OF A. A. A. S.

Wednesday afternoon, Dec. 26, 2.00 p.m.; Keystone Room, Hotel Statler. *Symposium: Genetics of the Fungi.* **Program of the Mycological Society of America, co-sponsored by A. A. A. S. Section G-Botanical Sciences.** Arranged by LINDSAY S. OLIVE, Columbia University, and ALMA W. BARKSDALE, New York Botanical Garden.

HAIG PAPAZIAN, 169 Cold Spring Street,
New Haven, Conn., *Presiding*

1. Evolution of Heterothallism in the Ascomycetes. HARRY WHEELER, Louisiana State University. (Lantern, 30 min.)
2. Discussion.
3. Nuclear Phenomena Associated with Heterocaryosis, Caryogramy, and Segregation in the Homothallic Ascomycete *Sclerotinia trifoliorum* Eriksson. ALEC J. H. CARR, University College of Wales, Welsh Plant Breeding Station, Aberystwyth, Wales. (Lantern, 30 min.)
4. Discussion.
5. Gene Conversion in Fungi. PATRICIA ST. LAWRENCE, Yale University. (Lantern, 30 min.)
6. Discussion.
7. Genetic Control of Mating-type in Fungi. HAIG PAPAZIAN. (Lantern, 30 min.)
8. Discussion.

COLONY MOUNTS OF FUNGI

Since the publication of my note on Colony Mounts of Fungi in Mycologia **48**: 448–450–1956 it has come to my attention that mention of

two papers bearing on the subject was omitted. These were by W. E. Sackston, Phytopathology **44**: 106–107. 1954 and by C. L. Duddington and S. M. Dixon, Nature **168**: 38–39. 1951—SCOTT V. COVERT, Albany Medical College, Albany, N. Y.

INCREASED CHARGE FOR STAIN CERTIFICATION

The Board of Trustees of the Biological Stain Commission has found it necessary to increase the price of Certification Labels by 5¢ effective September 1, 1956. This will result in a change from the current charge of 15¢ to a new charge of 20¢ per bottle to the purchasers of Certified Stains. This price increase has been necessitated by increasing costs in the operation of the Commission, and is the first such increase in almost 20 years.

MYCOLOGIA

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED JUNE 30, 1956

Cash receipts		
Joint funds		
Members' subscriptions (Mycological Society)	$ 3,622.50	
Other subscriptions	6,556.74	
Sales of V. 25 and later	809.86	
Excess pages and illustrations	908.96	$11,898.06
Special funds		
Sale of V. 1–24 and Index	688.52	
Income from endowment	905.00	1,593.52
Total receipts		$13,491.58
Cash disbursements		
Printing and distribution	$11,569.32	
Reprinting	613.55	
Office expense	404.67	
Total expenditures		$12,587.54
Excess of receipts over expenditures		904.04
Unexpended balance July 1, 1955		4,146.09
Unexpended balance July, 1956		$ 5,050.13
Endowment July 1, 1955	$14,000.00	
Proportionate share of profits from sales of securities	5,450.00	
Endowment July 1, 1956		$19,450.00

The above MYCOLOGIA funds are administered by The New York Botanical Garden, and the balances at June 30, 1956, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Company.

INDEX TO VOLUME XLVIII *

INDEX TO AUTHORS AND TITLES

Ajello, Libero. See West and Ajello 163

Alexopoulos, Const. J. See Wilson and Alexopoulos 685

Bakerspigel, A. *Haplosporangium* in Saskatchewan rodents 568

Bandoni, R. J. A preliminary survey of the genus *Platygloea* 821

Barnett, H. L., and V. G. Lilly. Factors affecting the production of zygospores by *Choanephora cucurbitarum* 617

Beneke, E. S. See Ringel and Beneke 329

Bonar, Lee. Elizabeth Eaton Morse 439

Bonner, John Tyler, K. Kent Kane, and Raphael H. Levey. Studies on the mechanics of growth in the common mushroom, *Agaricus campestris* 13

Bonorden, Roland. Growth of the yeast phase of *Histoplasma capsulatum* in simplified fluid medium 166

Brock, Thomas D. Lipid synthesis in *Hansenula anomala* 337

Butler, Edward E. Spore germination in *Sordaria fimicola* 345

Cantino, Edward C. The relation between cellular metabolism and morphogenesis in *Blastocladiella* 225

——, **and E. A. Horenstein.** Gamma and the cytoplasmic control of differentiation in *Blastocladiella* 443

——, ——. The stimulatory effect of light upon growth and CO_2 fixation in *Blastocladiella*. I. The S. K. I. Cycle 777

Carmichael, J. W. Frozen storage for stock cultures of fungi 378

——. The cellophane technique for studying morphology and hyphal fusions in fungi 450

Ciferri, Orio. See Gottlieb and Ciferri 253

Cochrane, Vincent W. The anaerobic dissimulation of glucose by *Fusarium lini* 1

Cooke, Wm. Bridge. The genus *Phlebia* 386

Covert, Scott V. Colony mounts of fungi 448, 885

Creelman, D. W. Spot anthracnose of linden 552

Critopoulos, P. Perpetuation of the brown rust of barley in Attica 596

Cummins, George B. Nomenclatural changes for some North American Uredinales 601

——. See Hennen and Cummins 126

Dick, Esther A. See Snell and and Dick 167, 302, 327

Drechsler, Charles. Supplementary developmental stages of *Basidiobolus ranarum* and *Basidiobolus haptosporus* 655

Ervin, Marion D. A new genus of the Tremellaceae 690

Fergus, Charles L. The influence of actidione on wood-staining fungi 468

* This index prepared by Eleanor Ruggeri Cioffi.

Fuentes, César A. A new species of *Microsporum* 613
——, **and Frederick A. Wolf.** The perfect stage of *Hormodendrum pedrosoi* 56
——, ——. *Microascus pedrosoi* is *M. cinereus*—a correction 446
Garner, J. H. B. Gasteromycetes from Panama and Costa Rica 757
Georg, Lucille K. Studies on *Trichophyton tonsurans*. I. The taxonomy of *T. tonsurans* 65
——. II. Morphology and laboratory identification 354
Goos, Roger D. On *Melanospora episphaeria* 881
Gordon, Morris A. Occurrence of *Tricellula inaequalis* in the United States 320
Gottlieb, David, and Orio Ciferri. Deamination and degradation of amino acids by streptomycetes 253
Groves, J. Walton, and Doreen E. Wells. The genus *Retinocyclus* 865
Hansen, H. N. See Zachariah, Hansen, and Snyder 459
Heidenhain, Berta. See Lowry, Sussman, and Heidenhain 241
Heim, Allen H., and Hubert Lechevalier. Effect of iron, zinc, manganese and calcium on the growth of various strains of *Streptomyces* 628
Hennen, Joe F., and George B. Cummins. Uredinales parasitizing grasses of the tribe Chlorideae 126
Horenstein, E. A. See Cantino and Horenstein 443, 777
Huxley, M. Joan. See Skinner and Huxley 371
Johns, Robert M. Additions to the phycomycete flora of the Douglas Lake region. III. A new species of *Scherffeliomyces* 433
Johnson, T. W., Jr. Marine Fungi. I. *Leptosphaeria* and *Pleospora* 495
——. II. Ascomycetes and Deuteromycetes from submerged wood 841
Kao, C. J. The cytology of *Xenogloea eriophori* 288
——. The cytology of *Syzygospora alba* 677
Kane, K. Kent. See Bonner, Kane, and Levey 13
Kaplan, Leo. A rotary shaking machine for laboratory use 609
Karling, John S. Undescribed species of *Synchytrium* 83
——. Development of *Synchytrium innominatum* in relation to other similar species 534
Kennedy, Lorene L. *Dacrymyces palmatus* 311
——. The names *Dacrymyces stillatus* and *Dacrymyces abietinus* 878
Korf, Richard P. Nomenclatural notes. I. Misuse of neotypes for *Venturia* and *Phaeosphaerella* 591
——. *Daleomyces, Durandiomyces,* and other sparassoid forms of operculate Discomycetes 711
Kuehn, Harold H. Observations on Gymnoascaceae. III. Developmental morphology of *Gymnoascus reessii,* a new species of *Gymnoascus* and *Eidamella deflexa* 805
Lange, Morten. A new species of *Picoa* 877
Leaphart, Charles D. Physiological studies of some fungi associated with pole blight of western white pine 25
Leathers, Chester R. New species and varieties of *Clavaria* from Michigan 278
Lechevalier, Hubert. See Heim and Lechevalier 628
Leppik, E. E. Some viewpoints on the phylogeny of rust fungi. II. *Gymnosporangium* 637
Levey, Raphael H. See Bonner, Kane, and Levey 13
Lilly, V. G. See Barnett and Lilly 617

Limber, Donald P. A note on *Sporonema pulvinatum* Shear 612
Lingappa, B. T. *Synchytrium borreriae*, an endophytic alga 427
Lowe, Josiah L. Type studies of the polypores described by Karsten 99
Lowry, R. J., A. S. Sussman, and Berta Heidenhain. Lysozyme-induced sensitivity of *Neurospora* ascospores to furfural 241
Lowy, Bernard. A note on *Sirobasidium* 324
Mains, E. B. The relationship of *Cudoniella* and *Helotium* 410
——. North American species of the Geoglossaceae. Tribe Cudonieae 694
Miles, Philip G., and John R. Raper. Recovery of the component strains from dikaryotic mycelia 484
Mishra, J. N. Ustilaginales from Bihar. I. Two new species 406
——. II. Five undescribed species 872
Mohan, R. R. See Schatz, Trelawny, Schatz, and Mohan 883
Morris, Everett F. Tropical Fungi Imperfecti 728
Nelson, Paul E., and Stephen Wilhelm. An undescribed fungus causing a root rot of strawberry 547
Page, Robert M. Studies on the development of asexual reproductive structures in *Pilobolus* 206
Parmelee, J. A. The identification of the *Curvularia* parasite of *Gladiolus* 558
——. See Savile and Parmelee 573
Paterson, R. A. Additions to the phycomycete flora of the Douglas Lake Region. II. New chytridiaceous fungi 270
Pfaff, H. J. See Shifrine and Pfaff 41
Plakidas, A. G. *Cercospora* leaf spot of *Abelia* 382
——. Note concerning *Cercospora abeliae* 880
Ragab, M. A. A contribution of the fungi of Egypt 167
Rangaswami, G. In vitro effect of mycothricin on plant pathogenic bacteria and fungi 800
Rao, P. N., M. A. Salam, and M. J. Thirumalachar. A new genus of downy mildew on sorghum 860
Raper, John R. See Miles and Raper 484
Raper, Kenneth B. Factors affecting growth and differentiation in the simple slime molds 169
Ringel, Samuel M., and E. S. Beneke. The influence of certain sugars on the antifungal activity of sodium pyridinethione 329
Rosinski, Martin A. Development of the ascocarp of *Anthracobia melaloma* 506
Routien, John B. The development of *Boudiera areolata* on soil in the laboratory 328
——. A slime mold in an unusual habitat 612
Salam, M. A. See Rao, Salam, and Thirumalachar 860
Savile, D. B. O., and J. A. Parmelee. Some fungal parasites of Portulaceae 575
Schatz, A., G. S. Trelawny, V. Schatz, and R. R. Mohan. Respiration of amino acids by *Streptomyces nitrificans* 883
——, **Vivian Schatz, and Gilbert S. Trelawny.** Antifungal properties of tetrazolium compounds 473
Schatz, Vivian. See Schatz, Schatz, and Trelawny 475
——. See Schatz, Trelawny, Schatz, and Mohan 883
Shifrine, M., and H. P. Pfaff. The association of yeasts with certain bark beetles 41

Singer, Rolf. New genera of fungi. VII 719
——. *Boletus luridus* in North America 768
——. The *Pleurotus-hirtus*-complex 852
Sinski, James T. Abortion reactions to infection by *Synchytrium brownii* 420
Skinner, C. E., and M. Joan Huxley. *Rhodotorula glutinis* 371
Snell, Walter H., and Esther A. Dick. Three Rhode Island polypores 167
——, ——. Notes on Boletes. IX 302
——, ——. An unusual phalloid from Massachusetts 327
Snyder, William C. See Zachariah, Hansen, and Snyder 459
Sparrow, F. K. Observations on chytridiaceous parasites on phanerogams. V. The occurrence of *Physoderma butomi* and *P. vagans* in the United States 765
Sprague, Roderick. Some leafspot fungi on western Gramineae. X 741
Steyaert, R. L. A reply and an appeal to Professor Guba 767
Sussman, A. S. See Lowry, Sussman, and Heidenhain 241
Thirumalachar, M. J. See Rao, Salam, and Thirumalachar 860
Trelawny, Gilbert S. See Schatz, Schatz, and Trelawny 475
——. See Schatz, Trelawny, Schatz, and Mohan 883
Vanbreuseghem, R. *Histoplasma duboisii* and large forms of *Histoplasma capsulatum* 264
Wang, Mrs. Stephen S. See Kao, C. J. 288, 677
Wells, Doreen E. See Groves and Wells 865
West, Billy, and Libero Ajello. The occurrence of *Arachniotus citrinus* in soil 163
Wheeler, H. E. Sexual versus asexual reproduction in *Glomerella* 349
Wilhelm, Stephen. See Nelson and Wilhelm 547
Williams, Marion E. See Ervin, Marion D. 690
Wilson, G. B., and Const. J. Alexopoulos. Spontaneous mutation in *Gelasinospora calospora*, a homothallic fungus 685
Wolf, F. A. See Fuentes and Wolf 56, 446
Yarwood, C. E. Simultaneous self-stimulation and self-inhibition of uredospore germination 20
Yerkes, William D., Jr. *Chaetoseptoria Wellmani* in Mexico 738
Zachariah, Anna T., H. N. Hansen, and William C. Snyder. The influence of environmental factors on cultural characters of *Fusarium* species 459

INDEX TO BIOGRAPHIES, REVIEWS, AND NOTICES

Albritton, E. C. (ed.). Standard Values in Nutrition and Metabolism; review 454
Bärner, J. Bibliographie der Pflanzenschutz-Literatur, 1951; review 614
Christensen, Clyde M. Common Fleshy Fungi, rev. ed.; review 456
Civil Service Announcement 770
Coudert, Jean. Guide Pratique de Mycologie Médicale; review 771
Garrett, S. D. Biology of Root-Infecting Fungi; review 772
Increased charge for stain certification 886
Johnson, Terry W. The Genus *Achlya*. Morphology and Taxonomy; review 774
Laboratory Refresher Training Course 769
Morse, Elizabeth Eaton; biographical note and bibliography 439
Murrill, W. A.; portrait and note 457

Mycological program at New York meeting of A. A. A. S. 885
Mycological Society fellowships 452
Naumov, N. A. The Fungus Flora of the Leningrad Region; review 614
A new microbiological journal [Giornale di Microbiologia] 168
The Gertrude S. Burlingham Fellowship 168

INDEX TO TAXA

New taxa and the pages where they appear are in **bold face type.** An attempt has been made to correct mistakes in the spelling of scientific names where such occur in the text.

Absidia 378
Acaulium 446; albo-nigrescens 446; nigrum 446
Achlya 774; subg. Achlya 775; subg. Centroachlya 775; subg. Subcentrica 775
Achroomyces 821, 822; effusa 835; fimicola 831; tiliae 821, 831; tumidus 831
Achrotelium **lucumae 601**
Acrasis 193, 198
Acrostalagmus 378
Acrotheca 550
Acrothecium 558; lunatum 558
Actinomucor repens 167
Actinomyces scabies 479
Acytostelium 179, 181, 196–198; **leptosomum 179,** 181, 196, 197
Aecidium banaticum 149; brandegei 146; calirhoes 141; cephalanthi 135; claytonianum 574; delphinii-consolidae 149; fraxini 131; gossypii 132, 141; hyalinum 603; hibisciatum 141; jalapense 605, 606; jamesianum 146; magnatum 159; malvastri 141; malvicola 141; marci 148; napaeae 141; obesum 135; opuntiae 151; plantaginis 148; polemonii 159; raciborskii 149; solms-laubachii 148; sphaeralceae 141; tissae 159
Aerobacter 196
Agaricus calyx 857; campestris 13, 455, 774; hirtus 853; jacksonii 856; pescaprae 722
Agyrium nigricans v. minus 821, 831
Albugo 861; ipomoeae-pandurinae 431; portulacae 588
Aleuria 711; proteana 715; proteana v. proteana 715; proteana v. sparassoides 714, 715; proteana v. slavkoviensis 715
Aleurisma 378
Aleurosporia acuminata 78; effracta 78; plicatilis 78
Allescheria 378
Allomyces 445, 446; cystogenus 167
Alternaria 378, 732; consortiale 732; maritima 842, 843; oleraceae 801–803
Amanita 719
Amauroascus 379
Anthracobia melaloma 506
Antrodia 100; serena 100; serpens 105; serpens v. tuber 100
Anthostomella 379
Aporpium caryae 120
Apostemidium 696
Arachniotus 379; citrinus 163
Arenariomyces 848; cinctus 848, 850; trifurcatus 848
Armillaria 725
Armillariella chrysophylla 726
Arrhytidia enata 838
Ascobolus citrinus 530; furfuraceus 530
Ascochyta 748; brachypodii 755; phleina 748, 749; sorghi 746, 748, 749, 751, 755
Ascocybe grovesii 468–472
Ascophanus carneus 530
Ascotremella 694, 698; turbinata 701
Aspergillus 379, 772; luchuensis 168; niger 10, 801
Auricula 386, 387, 401; strigoso-zonata 402
Auricularia 386, 401, 691, 821; aurantiaca 391; auricula 298; butleri 402; mesenterica 299, 401; persistens 401; reflexa 402; rugosissima 402; rugosissima f. resupinata 402; sordescens 402; strigoso-zonata 402; tenuis 402; tremelloides 400

Bacillus megaterium 250
Bacterium stewartii 801, 802
Baeomyces roseus 705
Basidiobolus haptosporus 655; lacertae 673, 675; meristosporus 657–659, 665, 668, 669, 671, 674, 675; myxophilus 675; ranarum 655
Basidiophora 861
Beauveria 379
Biatorella 865
Bitzea 602; ingae 602
Bjerkandera 100; acricula 100; alborosea 100; chionea 102; cinerata 100; ciliatula 100; colliculosa 101; fennica

101; irpicoides 101; melina 101; mollusca 101; pallescens 102; pellita 102; pura 102; roseomaculata 102, 110, 123; serpula 103; simulans 103; subsericella 103
Blastocladia 238
Blastocladiella 225, 443, 777; emersonii 225–238, 443–445, 777
Blastomyces 265, 379; dermatitidis 568, 569
Boletus erythropus 307; hypocarycinus 307; impolitus 308, 769; luteus 769; luridus 307, 308, 768; miniato-olivaceus v. subluridus 307; regius 309; subvelutipes 307, 308; variegatus 305
Botrytis 379
Botryosphaeria 826
Botryotrichum 379
Boudiera areolata 328
Bourdotia 690, 691; caesia 691; pululahuana 691; pululahuana ssp. caesia 690, 692; pululahuana ssp. galzinii 690
Brachysporium 731; trifolii 559, 562
Brauniella 720
Bubakia **erythroxylonis 601**
Bulgaria 456; inquinans 456

Cadophora brunnescens 469, 470; pini 469
Calocybe 725
Caeoma fraxinatum 131; fraxinites 131; hibisciatum 141; piperianum 602
Caromurus acuminatus 159; spartinae 159
Caloporus 103; expallescens 103; expallescens v. flavidulus 104
Caloderma petrianum 759, 762
Calvatia candida 757; cyathiformis 757; lilacina 757
Candida 42, 46, 265, 379, 380; albicans 449, 476–478; curvata 44, 46, 47, 50, 51, 53; mycoderma 44, 46, 48, 53; parapsilosis 44, 46, 47, 53; rugosa 44, 46, 48, 54; **silvicola** 44, 46, **47**, 50–54
Cantharellula 725, 726; subg. Pseudoarmillariella 725; subg. Pseudoclitocybe 725, 727; subg. Pseudoomphalina 725; subg. Pseudotricholoma 725, 727; cyathiformis 725; ectypoides 725
Cantharellus odoratus 735
Carbomyces 878
Catathelasma 725
Cephalosporium 379
Ceratocystis 26, 38, 39; pilifera 27, 29, 38, 39
Ceratosphaeria 845, 849, 850
Ceratostomella 42; ulmi 478
Cercospora 382; **abeliae 384**, 880
Cercosporella poagena 744
Chaconia 602; **ingae 602**
Chaetocladium 568
Chaetomium 379
Chaetoporus 115
Chaetoseptoria wellmanii 738
Chlamydoaleurosporia crateriformis 78
Chlorochytrium 429, 431, 432
Choanephora 379; conjuncta 617; cucurbitarum 222, 617
Chromocrea 379
Chrysomyxa **empetri 602**; **piperiana 602**
Cintractia eriocauli 408
Cincinella 379
Cladosporium 379; herbarum 730
Cladotrichum 730
Clavaria 278; botrytis v. **latispora** 279, **280**; **caulifloriformis 278**, 279, 282; **fuscoferruginea** 279, **281**; luteotenerrima v. **borealis** 279, **285**; **phycophila** 279, **286**; pulchra v. **globulina** 279, **285**; **rubicundula** 279, **283**, 284; rufescens 281
Clitocybe 719, 723, 725; calyx 857
Clitocybula 723, 726; abundans 723; cavipes 723
Coccidioides immitis 568, 569
Coenonia 198
Coilomyces schweinitzii 761
Coleopuccinia 646, 647
Coleosporium adenocaulonis 607; aridum 607; **eupatorii 603**; helianthi 298; sidae 298; spigeliae 608; vernoniae 298
Colletotrichum 349, 744; graminicola 744, 750; lagenarium 330, 334; lindemuthianum 353; phomoides 330–335
Collybia 723; velutipes 484, 486
Coprinus 379; lagopus 13, 14
Coralliochytrium 433, 436–438; scherffelii 436–438
Cordana pauciseptata 730
Coriolus 103, 104; applanatus 104; simulans v. borealis 104; sulcatus 104; velutinus 104; velutinus v. nigrescens 104
Corticium 389, 827; sect. Ceracea 389; effuscatum 486, 490, 491; ferax 826; hepaticum 402, 404; lividum 389, 404
Cortinarius 456
Corynella atrovirens 865
Cronartium **fusiforme 603**
Cryptococcus 46, 51, 379; diffuens 44, 51, 54; neoformans 44, 51, 54
Cucullaria 697
Cudonia 410, 411, 417, 418, 696, 697, 704, 705; circinans 702, 704–709; confusa 706; constrictospora 705, 709; grisea 705, 708, 709; helvelloides 707; japonica 707; lutea 696, 704–709; monticola 705, 707–709; ochroleuca 704, 705; osterwaldii 709; queletii 411, 412, 414; spathulata 705
Cudonieae 697

Cudoniella 410, 696, 697; acicularis 412–414, 418, 696; aquatica 410, 411; borealis 415, 416; marcida 410; muscorum 415, 416; queletii 410–414, 696; stagnalis 410, 411
Cunninghamella 379
Curvularia 558; andropogonis 559; geniculata 469, 730; lunata 558–567, 731; trifolii 558–567; trifolii f. sp. **gladioli** 560, 563, **566**
Cyathus poeppigii 487, 490, 673; stercoreus 487, 763
Cylindrosporium 743; **andropogoni 741**, 742
Cystoderma 719
Cystopsora 637; pini 469

Dacrymyces 313, 316, 317, 827, 878; abietinus 878; albidus 317; aurantius 311; deliquescens 878–880; palmatus 311; rubiformis 318; stillatus 878
Dacryomitra 313, 316, 319; depallens 318, nuda 314, 316; ramosa 311, 318; stipitata 316
Dacryomyces 878; abietinus 879; aurantius 318; chrysospermus 318; flabellus 318; multiseptatus 318; stillatus 878–880
Dacryopinax 316, 319; elegans 316; spathularia 315
Dacryopsis 311; palmata 311, 318
Daedalea 105, 115; betulina v. porosa 105; candicans 105; confragosa 105, 109, 121; oudemansii v. fennica 105; unicolor 105, 109; variegata 105
Daedaleopsis 105; incana 105
Daleomyces 711; gardneri 713, 715, 716; phillipsii 714; shearii 715, 716
Davisiella elymina 747
Dematium nigrum 196
Dematophora glomerata 63
Dennisiomyces 722–726; glabrescentipes 722; griseus 722
Dermoloma 724–726; atrobrunneum 724; cuneifolium 724
Desmella 637; obovata 608
Diaporthe phaseolorum v. batatatis 464
Dicaeoma 126; bartholomaei 146; boutelouae 144; campulosi 161; chloridis 146; cynodontis 148; diplachnis 146; distichlidis 142; enteropogonis 129; exasperans 140; guaraniticum 133; gymnopogonis 144; hibisciatum 141; kansense 143; jalapense 605; jamesianum 146; leptochloae 150; muhlenbergiae 141; plucheae 606; schedonnardi 141; triodiae 141; varians 149; vexans 139
Dicksonomyces 863; sorghi 863
Dictyoploca holoporphyra 720
Dictyostelium 172, 177, 179, 190, 193, 196; discoideum 171–198; lacteum 179, 191, 196, 198; minutum 179, 187, 191, 193, 196, 201; mucoroides 172, 175, 182, 186, 188, 190, 191, 199, 201; **polycephalum 175,** 179, 183, 186, 187, 192, 195; purpureum 172, 181, 186, 191, 199
Diorchidium boutelouae 144
Diplodia orae-maris 843, 844
Diplophlyctis laevis 273
Diplosporium album 729
Discina ancilis 717; convoluta 717; leucoxantha 717; perlata 717
Disciotis venosa v. reticulata 717
Ditiola 313, 316, 319, 879; radicata 314
Durandiomyces 711; phillipsii 714

Eidamella 815; deflexa 805; papyricola 816, 819, 820; spinosa 815, 816, 819, 820
Ellisiella caudata 744
Elsinoë 552, 553, 555; **tiliae 555**
Endoconidiophora fimbriata 469; moniliformis 469
Endomyces 379; bisporus 41
Endomycopsis 41, 379; bispora 41; guilliermondii 375
Endophyllum **lacus-regis** 577–579, 585, 589
Endothrix acuminatum 78; crateriforme 78
Endostigme 591–595
Endothrix sulfureum 78
Epicoccum purpurascens 168
Epidermophyton 379; floccosum 477
Erwinia atroseptica 801, 802
Erysiphe graminis 755
Escherichia coli 185, 342, 800
Eugymnosporangium 646–650
Exidia 690, 691; alba 691, 692; cartilaginea 691; glandulosa 691; nucleata 691; recisa 691; saccharina 691; tenuis 402; villosa 691
Exidiopsis alba 690, 692

Favolus alveolaris 487
Favotrichophyton 77; floriforme 78
Fayodia subg. Clitocybula 720
Femsjonia 316; luteoalba 316–319
Fomes 106; abietis 106; annosus 114; cajanderi 106, 121; igniarius 122; pini 106; pinicola 100; robustus 106, 122; roseus 492; subroseus 492; tenuis 106; thelephoroides 107
Fusarium 1, 8, 320, 459, 550; episphaeria 461–466; equiseti 168; lateritium 461–466; lini 1, 801; moniliforme 461–466, 478; moniliforme v. subglutinans 745; nivale 459, 745; oxysporum 9, 461–466, 733; oxysporum f. lini 2; oxysporum f. lycopersici 801; rigidiusculum 461–466; roseum 461–466; solani

9, 461–466; tricinctum 460–466; tricothecioides 1, 8
Fusicladium 593

Galactinia 711, 712; proteana 715; proteana v. sparassoides 714; proteana f. sparassoides 715; tosta 716
Geaster 456
Geastrum fimbriatum 761; mirabile 761; rufescens 761; saccatum 761; schweinitzii 761; triplex 761; velutinum 760
Gelasinospora 379; calospora 685; calospora v. autosteira 685
Geoglosseae 696
Geomyces 379; auratus 379
Geotrichum 168, 379, 772; candidum 451
Gerronema 723
Gibbera 592–595; vaccinii 593, 595
Gibberella 462; fujikuroi 167
Glococercospora 743; alascensis 742, 743
Glocoradulum 401
Glocosporium 349; meinersii 742, 745
Gloeotromera 692; alba 692; pulula-huana 692
Gliocladium penicillioides 728
Glomerella 349; cingulata 349
Gonytrichum macrocladum 730
Graphium rigidum 469, 732
Grosmannia 26
Guepiniopsis 316, 879; chrysocomus 879
Guttulina 172
Guttulinopsis 172
Gymnoascus 805; **brevisetosus 813**, 814, 817, 819; reessii 805; setosus 807, 808, 815
Gymnosporangium 637; sect. Bermudiani 645, 648; sect. Biseptatae 638, 644, 648; sect. Clavariiforme 645, 648; sect. Clavipes 638, 644, 648; sect. Communis 645, 648; sect. Confusum 645, 648; sect. Corniculans 646, 648; sect. Cupressi 638, 644, 648; sect. Ellisii 638, 644, 648, 649; sect. Eygymnosporangium 638, 643, 644, 646–650; sect. Eutelium 638; sect. Exterum 645, 648; sect. Globosae 646, 648; sect. Gymnotelium 638, 643, 646–649; sect. Haraeanum 645, 648; sect. Japonicum 645, 648; sect. Juniperinum 645, 648; sect. Juniperi-virginianae 648; sect. Libocedri 638, 644, 648, 649; sect. Miyabei 638, 644, 648; sect. Multiporae 638, 645, 648; sect. Nidusavis 645, 648; sect. Nootkatense 638, 644, 648, 649; sect. Oxycedri 638; sect. Sabinae 638, 646, 648; sect. Speciosae 638, 644, 648; sect. Trachysorum 645, 648; sect. Yamadae 645, 648; amelanchieridis 645; aurantiacum 645; bermudianum 646; betheli 642, 646; biseptatum 639, 641, 644; clavariiforme 642, 645; clavipes 642, 644; confusum 639, 645; corniculans 642, 646; cunninghamiana 641, 644; cupressi 641, 644; ellisii 639, 641, 644; exiguum 645; exterum 641, 645; globosum 641, 646; guatemalianum 606; haraeanum 639, 641, 645; harknessianum 639, 645; **hyalinum 603**, 644; inconspicuum 641, 644; japonicum 641, 645; juniperi-virginianae 642, 646; juniperinum 639, 641, 642, 645; kernianum 645; libocedri 639, 640, 644; miyabei 641, 644; multiporum 645; nelsoni 639, 646; nidusavis 641, 645; nootkatense 640, 644, 648, 649; sabinae 639, 646; speciosum 644; torminali-juniperinum 645; trachysorum 641, 645; transformans 639, 641, 644; tsingchenensis 648; yamadae 645
Gymnotelium 643, 646–649
Gyromitra 711; gigas 715; phillipsii 714, 715

Halophiobolus 847, 848; medusa 849
Hansenia 107; imitata 107
Hansenula 41–47, 52, 376, 377, 379; anomala 337; beckii 41; capsulata 44, 50–53; saturnus 478; silvicola 46, 47
Hantzschia 26
Haplosporangium 379, 568; parvum 568
Heimiomyces 722, 723; brunneipes 723; pruinatipes 723
Helicobasidium 821; fimetarium 831
Helicogloea 298, 821; lagerheimi 299, 838
Helicoma salinum 842, 844
Helminthosporium 746; avenae 801; carbonum 330, 334; catenarium 745, 746; dictyoides 746; gramineum 464, 746; portulacae 589; sorokinianum 743; stenacrum 746, 755; tritici-repentis 746; vagans 746
Helotium 410, 696–698; aciculare 411–418, 696; agariciforme 412, 414; **boreale 415**–417; citrinum 412; clavus 416; cudonioides 413–415; fimetarium 412; herbarum 412
Helvella 717; acicularis 412
Hemiglossum 694
Hendersonia 742, 747; culmicola 742, 747, 748; culmicola v. minor 748
Hendersonula aristidae 747
Herpobasidium 838
Hirneola tenuis 402
Histoplasma 379; capsulatum 163–166, 264, 568; duboisii 264
Hormiscium 842, 843, 850; gelatinosum 469
Hormodendrum 57, 61, 379, 446; compactum 56; pedrosoi 56, 446
Humaria granulata 525

Hyalopsora 298; aspidiotus 294, 299; obovata 608
Hydnum 386
Hydropus 722, 723; marginatus v. rugosodiscus 723
Hygromitra 697
Hymenochaete 401; tabacina 401

Idriella 550; lunata 548–**550,** 551
Inonotus 107; fulvus 107; levis 107; radiatus v. scrobiculatus 107; sulphureopulverulentus 108; triqueter v. purpurascens 108; ufensis 108; vulpinus v. hisingeri 108
Irpex 113
Ischnoderma dryadeum 106
Ithyphallus 456

Jola javensis 299

Kriegeria eriophori 288

Lachnea cretea 529, 530
Lactarius 456
Lactobacillus casei 212, 213; lactis 212
Leccinum aurantiacum 308; chromapes 309
Lentinellus 726, 727
Lentinus calvescens 855; nigro-osseus 853; vellereus 854
Lentodiellum concavum 856
Lenzites 108, 115; abietina 108; ambigua 108; betulina 105, 109, 486, 489, 490; laricina 108; pinicola 108; septentrionalis 109; sibirica 109; sibirica v. unizonata 109; sorbina 109; tricolor 109
Leotia 410, 411, 418, 694–698; acicularis 411; **albiceps** 695, 698, **700,** 702, 703; atrovirens 695, 698–700; chlorocephala 699, 700; circinans 697, 706; lubrica 411, 695–703; lubrica f. lloydii 699; lubrica f. stevensoni 699; lutea 706; punctipes 698; queletii 411, 414; stevensoni 700; stipitata 700; viscosa 695, 698–700
Leptographium 25–27, 37–40; engelmanni 469; lundbergii 26, 469
Leptoporus stipticus 100
Leptosphaeria 495, 749, 841; subg. Euleptosphaeria 503; subg. Scleropleela 503; albopunctata 495, 497–502, 505; avenaria 749; chondri 495; discors 495–498, 500, 502, 505; dryadis 503; dumentorum 503; galiorum 503; **halima** 501, **502**–505; herpotrichoides 749; incarcerata 495, 499, 500, 505; marina 495, 497, 498, 505; maritima 498–505; orae-maris 495, 497, 498, 502, 505; poae 503; scirpi 498; spartinae 495, 499, 500, 505; sticta 495, 499; tenera 503; typharum 503; vagans v. scirpi 498
Leptothyrium macrothecium 613
Leucopaxillus 721, 726; subg. Leucopaxillus 727; spinulosus 727
Lipomyces starkeyi 375
Lulworthia 847, 848; longispora 849; medusa 845, 848, 849; salina 845, 849
Lycogala 760
Lycogalopsis 760; dussi 760; solmsii 760; subiculosa 760
Lycoperdon 760; cepaeforme 758; curtisii 758, 763; ericetorum 758, 763; furfuraceum 758; molle 758, 763; perlatum 758; pusillum 758; spadiceum 758, 763; subincarnatum 757; umbrinum 758; wrightii 758

Macrophoma phlei 749
Madurella 379
Marasmius pallipes 853
Masonia 447; grisea 447
Massaria 846
Massariella 846; **maritima** 842, **846,** 850
Melampsora 647; piscariae 608
Melampsoropsis empetri 602; piperiana 602
Melanoleuca 721, 727; grammopodia 721
Melanospora 379, 882, 883; episphaeria 881; stysanospora 447; zamiae 168
Menispora 550; cobaltina 550
Merulius 386, 389, 401; sect. Coniophori 389; sect. Leptospori 389; fulvus 391; merismoides 391; serpens 111; strigoso-zonatus 401, 403
Metasphaeria australiensis 849; discors 495, 496
Microascus 61–63, 446, 447; cinereus 446; cirrosus 62, 63; intermedius 62; longirostris 62; lunasporus 61–63; nidicola 62; **pedrosoi** 58, 59, **63,** 446; sordidus 62; trigonosporus 61–63, 477; variabilis 62
Micrococcus pyogenes v. aureus 329
Microglossum 694, 697
Micropuccinia rotundata 606
Microsporum 379, 613; audouini 356, 476, 477; canis 356, 477, 478; gypseum 163, 165, 613, 614; **nanum 614**
Milesia consimilis 607
Monascostroma 593
Monascus purpureus 167
Monilia 42, 379
Monochaetia 767
Monosporium 379
Morganella mexicana 759, 760
Mortierella 379
Mucor 379; hiemalis 167
Mutinus caninus 327
Mycena 719; sect. Purae 720; pura 720
Mycobacterium avium 329

Mycogloea 821
Myrothecium roridum 732
Myxotrichum 379, 805; conjugatum 805; deflexum 816–820; emmonsii 805; thaxteri 805; uncinatum 805

Naematelia rubiformis 318
Napomyces 711, 713; gardneri 715
Naumoviella 615
Neocudoniella jezoensis 703
Neogyromitra 717
Neotrichophyton flavum 78; plicatile 78
Neottiosporella 735; **radicata** 734, **735**, 736; triseti 736
Neottiosporis 736
Nephrospora mangini 447
Neurospora 241, 379; crassa 10; tetrasperma 241, 242, 251, 379
Nigredo acuminata 159; archeriana 155; arguta 152; polemonii 159
Nigrospora 168
Nocardia 379

Obelidium 273
Ocellaria betuli v. nigrescens 831
Odontia 827; lateritia 401
Oidium tonsurans 67, 78
Ombrophila 694, 698, 701; albiceps 698, 700, 701, 703; clavus 411, 416, 698; violacea 698
Omphalia kalchbrenneri 725
Omphalina 723
Oospora 379
Ophiobolus medusa 849
Ophiostoma 472; adiposum 469, 470; canum 469; coeruleum 469, 470; ips 469; majus 469; multiannulatum 469; picea 469, 471, 472; piliferum 469; pini 469; pluriannulatum 469; ulmi 469
Ovularia 593; pusilla 745

Pachycudonia 705; monticola 707
Paecilomyces 379; varioti 728
Palomyces 848; quadri-remis 848
Panus 853; concavus 856; hirtus 852, 853; infundibulus 854; rudis 853; tigrinus 857, 858
Passeriniella 498, 500
Patella 506
Patellina fragariae 613
Patouillardina cinerea 838
Penicillium 447, 667, 772; brefeldianum 168; chrysogenum 10; notatum 10
Peniophora 389, 683, 827; coccineofulva 400; livida 683
Periconia 729; byssoides 729; cambrensis 729; circinata 729; igniaria 729
Peridermium fusiforme 603; parksianum 602
Peristomium desmosporum 63, 447
Peritrichospora integra 843, 848, 850
Peronospora 860, 861, 863; claytoniae 586
Pestalotia 733, 767, 768; dichaeta 733; disseminata 733; pezizoides 733
Pestalotiopsis 733, 767, 768; dichaeta 733; disseminata 733
Peziza 711, 712, 716; subg. Galactinia 712, 713, 716; abietina 878, 880; aciculoris 411, 414; oenotherae 613; phillipsii 713, 714; proteana 711–716; proteana v. sparassoides 714; proteana f. **proteana 715**; proteana f. **sparassoides 714–717**; **shearii 716**; sparassiformis 716; tjibodensis 716; vesiculosa f. saccata 716
Phaeophlebia 390, **401**; **strigoso-zonata 401**
Phaeoscopulariopsis 447; bestae 447; paisii 447
Phaeoseptoria festucae 750, 753
Phaeosphaerella 591; macularis 592, 593; maculosa 592, 593; typhae 593
Phakopsora **antiguensis 604**; **cherimoliae 604**; **jatrophicola 604**
Phialea 746
Phialophora 379; verrucosa 56, 57, 730
Phlebia 386; sect. Corticioideae 384; sect. Phlebia 389; sect. Merulioideae 389; acerina 391, 392; albida 388, 390, 391, 395, 405; americana 397; anomala 402, 403; **argentinensis** 390, **394**, 405; **atkinsoniana** 390, **393**, 405; aurantiaca 391; aurantiaca v. contorta 393; blumenaviensis 400; **canadensis** 390, **398**, 405; candida 397; castanea 401; **celtidis** 390, **394**, 404; centrifuga 388, 395; centrifuga v. cervina 397; cervina 400; cinnabarina 388, 391–393; coccineo-fulva 400; contorta 387, 388, 391, 395; coriacea 400; **cystidiata** 390, 393, **395**, 404; deglubens 399; depallens 393; donkii 389, 391, 392; erecta 400; hispidula 402; hydnoidea 401; kreigeriana 391, 395; lirosella 401; macra 395, 397; macra v. roseo-inhalata 395; macra f. roseomarginata 395; marginata 397; mellea 395–397; melleopallens 397; merismoides 387–395; merismoides f. tenuior 393; merulioides 401; mesenterica 401; moelleriana 400; **murrillii** 390, **398**, 404; orbicularis 402; pileata 402, 403; radiata 387–395, 404; radiata α carnea 391; radiata ζ carneo-plumbea 391, radiata γ cinnabarina 391; radiata δ fulgens 391; radiata ε livida 391; radiata v. microspora 393; radiata β pallida 391; radiata f. cerasi-avium 391; reflexa 402; rubiginosa 402, 403; rugisissima

402; sodiroi 399; sordida 400; spilomea 402; strigoso-zonata 401, 402; **subalbida** 390, **397**, 405; tremelloides 400; vaga 400; zonata 402, 403
Phlebiella vaga 400
Phlyctochytrium 273, **437**; dentatum 276; mucronatum 273; **unispinum 270**, 271, 273, 274; zygnematis 276
Phycomyces 379; blakesleeanus 212, 618
Phyllachora 747; graminis 749
Phyllodontia 109; magnusii 109
Phyllosticta 743, 752; anthoxella 760; bromivora 743; **digitariae** 742, **753**; rogleri 743
Physalospora 842, 847, 848, 850
Physisporus 110; alboater 110; albolilacinus 102, 110; aurantiacus v. saloisensis 101, 110, 122; aurantiacus v. taloisensis 110; caesio-albus 110; crassus 110; eupatorii 111; flavicans 111; fragillimus 112; inconstans 112; lacer 113; lenis 113; locellatus 113; luteoalbus 112, 113; makraulos v. nigromarginatus 114; microsporus 114; molluscus * bombycinoides 114; nitidus 114, 116; tener 115; tuber 115; variecolor 114, 115; vitellinulus 114, 116; vitellinus 114–116
Physoderma 95, 97, 587; butomi 765; claytoniana 587–589; claytoniana v. **sparrowii 587**–589; pluriannulata 95, 97; vagans 765
Physopella cherimoliae 604
Physospora 861
Pichia 44, 45, 379; **haplophila** 44, **45**, 52, 53
Picoa, 877; juniperi 878; lefebvrei 878; **pachyasmus 877**
Pileolaria extensa 607; mexicana 608
Pilobolus 206; crystallinus 220; kleinii 207, 209, 219, 220, 222
Platycarpa 838
Platygloea 82, 288; arrhytidiae 823, 826, 827, 838; blastomyces 824, 833; brunnea 823, 827; carnea 823, 829; caroliniana 822, 824, 834; cissi 824, 836; decipiens 824, 828, 836; disciformis 821, 823, 825, 831; effusa 824, 835; eriophori 288, 838; fibrosa 823, 832; fimetaria 823, 831; fimicola 831; fusco-atra 826, 838; grandinia 838; hymenolepidis 838; javanica 823, 827; lagerheimii 838; lagerstroemiae 824, 834; longibasidia 824, 833; micra 824, 835; miedzyrzecensis 823, 824; minima 838; nigricans 299, 821, 822, 831; peniophorae 823, 826, 830; pini 838; pustulata 821, 824, 828, 833; sphaerospora 824, 831, 836; subvestita 824, 830, 837; succinea 823, 829; tiliae 831; unispora 823, 828, 832; vestita 824, 831, 837
Pleospora 495, 592, 841; laminariana 495, 504; maritima 495, 504, 505; **pelagica** 501, **504**, 505; pelvetiae 495, 504
Pleurotus 852, 853; **calvescens** 853, **855**, 858, 859; **calyx** 853, **857**–859; **concavus** 853, 855, **856**, 858, 859; dryinus 857, 858; hirtus 852; ostreatus 487, 490, 852, 853
Plicaria 716
Podoporia 116; confluens 116
Polypilus 116; conglobatus 116
Polyporellus 116; albulus 116; brumalis f. trachypus 117; caudicinus 117; hisingeri 117; lepideus 117; lepideus v. submurinus 117; leptocephalus v. terrigenus 117; melanopus 117; sororius 117; tubaeformis 117; varius 117
Polyporus 117, 123; abietinus 108, 110; adustus 103, 105, 121; albellus 100; albolutescens 123; amorphus 100–102, 118; amorphus v. albus 117; anceps 123; betulinus 487; biennis v. distortus 116; borealis 101; borealis v. spathulata 122; brumalis 117; collabens 118; contiguus 118; dichrous 103, 104; dryophilus 107; caesius 100; euporus 118; fagicola 117; fibrillosus 118; flavus 119; floriformis 103, 116; fomentarius 119; fragilis 103; fumosus 101, 102; griseus 167; hians 119; hirsutus 104; holmiensis 102; imberbis 101, 102; immitis 100, 101; inonotus 108; isabellinus 106; karstenii 103; lacteus 100, 101; melanopus 117; mollis 100, 108; osseus 102; pallescens 119; picipes 117; pubescens 104, 107; radiatus 103, 107; rheades 107; rhodellus 118, 121; rixosus 118; rostafinskii 119; rutilans 122; schweinitzii 108; selectus 119; semisupinus 113, 119; sericeomollis 113; sommerfeltii 120; squamosus 117; stipticus 100, 101; sulphureus 117, 167; sulphureus v. semialbinus 167; tephroleucus 101, 103; tomentosus 108; trabeus 101; tulipiferae 113; varius 117; versicolor 487, 490; vulgaris 119; vulpinus 107
Polysphondylium 172, 179, 191, 192, 196; pallidum 192; violaceum 172, 176, 177, 186, 188
Poria 112, 120, 827; albobrunnea 123; aneirina 100; aurantiaca 110; biguttulata 114; blytii 118; bombycina 119; calcea 113, 114; candidissima 112, 115, 123; canescens 120; carbonicola 120; corticola 115, 119; cupora 115; europaea 115; fatiscens

111; ferrea 106; ferrugineofusca 120; flavicans 112; labyrinthica 120; lenis 113, 115; loennbohmii 121; luteoalba 114, 115; medulla-panis 16; micans 118; microspora 102, 492; mollusca 112, 114; monticola 102, 110; nigrescens 114; nitida 110, 118; oleagina 111; pannocincta 116; pulchella 115, 116; resinascens 121; reticulata 104; sanguinolenta 104, 110, 116, 121; separabilis 121; sericeomollis 112, 113; **squalens 123**; starbaeckii 121; subacida 115; unita 115, 116; vaillantii 114; viridans 112; vitellinula 116; vulgaris 113; xantha 111, 114, 115, 119
Poromycena 720; violacella 720
Poronidulus conchifer 486
Porpoloma 720–726; subg. **Pes-caprae 722**; subg. **Porpoloma 722**; pescaprae 722; portentosum **722**; sejunctum 722; terreum 722
Proto-Gymnosporangium 638, 643
Protomyces macrosporus 431
Protoventuria 592, 593, 595
Pseudoarmillariella 725, 726; **ectypoides 725**
Pseudoclitocybe 725, 727; **cyathiformis 725**
Pseudocolus shellenbergiae 327
Pseudogymnoascus 379
Pseudohydnum guepinioides 400
Pseudoomphalina 725, 726; **kalchbrenneri 725**
Ptychogaster rubescens 487
Ptychoverpa bohemica 717
Puccinia 126, 128, 131, 161; sect. Bullaria 144; agnita 574, 575, 585, 589; **ammophilina 604**; anomala 596; aristidae 161; aristidicola 139; bartholomaei 138, 140, 145, 146; beckmanniae 127; **biocellata 605**; boutelouae 129, 135, 140, 144–148; buchloes 161; cacabata 128, 130, 132, 133, 141; campulosi 161; cephalanthi 135; chichenensis 133, 134; chloridicola 136, 138; chloridina 136, 138; chloridis 129, 136, 138–140, 145–147; chloridis-incompletae 129, 131; claytoniae 580; claytoniicola 579, 580, 585, 589; coronata 127; **costina 605**; cynodontis 126, 129, 145, 148, 150, 160; dactyloctenii 136, 138; **degener 605**; desmazieresi 161; dieteliii 128, 136–138, 147, 148; diplachnis 146; distichlidis 128, 137, 142, 143; enteropogonis 128–130; epicampes 141; exasperans 128, 133, 137, 140, 146; fraxini 131; gentianae 608; graminis 20, 127, 639, 641; graminis v. tritici 20; helianthi 23; guaranitica 128–130, 133, 134, 145; **gymnopogonicola** 128, 130, **134**, 135, 147, 148; gymnopogonis 135, **144**, 146; hibisciata 141; hordei 596; jalapensis 605; jamesiana 146; kansensis 126, 128, 137, 143; kelseyi 142; leptochloae 129, 145, 150, 151; lojkaiana 599; mariae-wilsoniae 573–579, 588, 589; mariae-wilsoniae v. **mariae-wilsoniae 573**, 585; mariae-wilsoniae v. **montiae 574**, 585, 589; moliniae 161; muhlenbergiae 141; **neorotundata 606**; opuntiae 129, 133, 145, 151; peridermiospora 131; plucheae 606; poae-nemoralis 161; poae-sudeticae 161; rotundata 606; rugosa 606; schedonnardi 128, 137, 141, 142; seymouriana 128, 130, 132, 135, 143; simplex 596; sorghi 330, 334; sparganioides 128–132, 136, 143, 154; spegaziniella 141; stakmanii 132, 133; subglobosa 141; subtilipes 129, 148; trichloridis 145, 147, 148; triodiae 141; varians 149; vexans 128, 133, 137–141; windsoriae 141
Pucciniastrum alaskanum 607
Pycnoporus 121; mimicus 106, 121; palibini 121
Pyrenophora 592
Pyronema confluens 516, 528, 531

Radulum 386
Ramularia 549, 550
Ramulispora 743
Ravenelia cubensis 607; hoffmanseggiae 608; inquirenda 607
Remispora maritima 845, 849
Retinocyclus 865; **abietis** 867, **869**–871; olivaceus 865–871
Rhizidium 276
Rhizina undulata 506, 530
Rhizoctonia choussii 168; solani 744
Rhizophydium digitatum 276; hyalothecae 276; **pedicellatum 274**–276
Rhizopus 379; nigricans 10
Rhodochytrium 429; spilanthidis 427, 431
Rhodotorula 46, 49; aurantica 375; colostri 375; **crocea 44**, 49, **50**, 52, 54; glutinis 371; glutinis v. rubescens 372; minuta 372, 373, 376; mucilaginosa 371–376; pallida 372; rubra 371–373, 376; sanguinea 371
Rhynchosporium 754; secalis 749
Rienophora carnea 391
Roestelia hyalina 603; **guatemaliana 606**
Rosellinia schumacheri 447
Russula 456; emetica 683

Saccharomyces 44, 45, 342, 379; cerevisiae 455, 478; pastori 52, 53; **pini 44**, **45**, 50–54

Saprolegnia 775
Sarcoporia 122; polyspora 122
Scherffelia 437
Scherffeliomyces 433; appendiculatus 434, 436, 437; **leptorrhizus 433–438**; **scherffelii 437**, 438; parasitans 434
Schizophyllum 485, 491; commune 399, 487, 488, 490
Schizosaccharomyces 298, 379; octosporus 294, 299
Schizotrichella 733, 735, 736; **lunata** 734, **735**, 736
Schizotrichum 735
Scleroderma 762; cepa 762; chrysastrum 762; lycoperdoides 761, 762; verrucosum 759, 761, 762
Sclerophoma entoxyli 469, 470
Sclerospora 860, 861, 863; sorghi 860
Sclerotinia fructicola 801–803; trifoliorum 885
Sclerotiopsis concava 613
Scolecotrichum graminis 744
Scopella **bauhiniicola 606**; **sapotae 607**
Scopularia 26
Scopulariopsis 61, 379, 446, 447, 772; brevicaulis 446; cinerea 63, 446; lunasporus 447; trigonosporus 447
Sebacina 690, 691; subg. Bourdotia 691; galzinii 691; pululahuana 690, 691, 693
Seismosarca 690, 691; alba 690–692; hydrophora 690–692
Selenophoma bromigena 753; donacis 754; donacis v. linearis 742, 754; donacis v. stomaticola 754; obtusa 754
Sepedonium 379
Septogloeum dimorphum 288; oxysporum 755
Septoria anthoxanthina 742, 750; avenae 751; bromi 752; glyceriicola 750, 751; graminum 751; jaculella 752; oudemansii 751; poliomela 750, 751; nodorum 742, 747–751; tenella 742, 752
Serpula 386, 401
Serratia marcescens 175
Sirobasidium 324; albidum 324, 327; brefeldianum 324, 327; cerasi 324; magnum 324, 327; sanguineum 324, 325, 327
Skierka 637
Sordaria 379, 848; fimicola 167, 345, 379, 687
Sorosporium **apludae 875**; **leersiae** 875, **876**
Spathularia 696, 705; velutipes 696
Speira pelagica 844
Spermospora subulata f. ciliata 744
Sphaceloma 552, 555
Sphacelotheca isachnes 406; ischaemicola 872; **ischaemi-rugosi 872**, 875; **monakai 406**, 407
Sphaerellopsis 595
Sphaeria albopunctata 499; incarcerata 499; macularis 592; maritima 495, 500
Sphaeroderma 882, 883; episphaeria 881; thelebloides 882, 883
Sphaerulina 842, 847, 850; intermixta 847; orae-maris 847; **pedicellata** 843, **846**, 847, 850; vulpina 847
Spicaria divaricata 168
Spilocaea 593
Spilosticta 591–595; macularis 592
Spondylocladium 731; obovatum 731; xylogenum 731
Spongipellis 122; ambiens 122
Sporobolomyces 379
Sporodesmium bakeri 732
Sporonema 612; oxycocci 612; pulvinatum 612
Sporotrichum 379; schenkii 476–478
Stachybotrys atra 730
Stagonospora bromi 748; foliicola 750; simplicior 749, 753; vexatula 753
Stemonitis webberi 612
Stemphylium 379, 732, 844; botryosum 844; codii 844; consortiale 732; lanuginosum 844; **maritimum** 843, **844**, 845, 850; sarcinaeforme 844
Stereum 401, 404; ceriferum 402; hispidulum 402; lugubre 402; pini 400; purpureum 487
Stictis tiliae 831
Stilbella 732
Stilbum vulgare 682
Streptomyces 377, 628, 656, 658; aurcofaciens 628–632; coelicolor 6, 10, 628–632; diastaticus 254; faveolus 628–632; fradiae 628–635; griseus 253, 254, 628–632; lavendulae 253–257, 261, 262, 628–632, 800; nitrificans 883; rimosus 628–632; venezuelae 254–262
Strobilomyces 456
Stysanus 446, 447
Suillus 303, 305; acidus 303; cothurnatus 303; luteus 303, 304; **megaporinus 302**; **pinorigidus 304**; subluteus 303; **subvariegatus 306**; variegatus 305
Syncephalastrum 379
Synchytrium 83, 420, 427, 429, 431, 534, 539, 541–544, 588, 589; subg. Eusynchytrium 540, 546; subg. Pycnochytrium 86, 90, 92–94, 97; subg. Pycnochytrium subgroup Chrysochytrium 95; aecidioides 540; athyrii 94; aureum 86–89, 91; australe 421, 534; borreriae 427; brownii 420, 431; decipiens 540; echii 85, 94, 97; endobioticum 420, 421, 425; epilobii 545; fulgens 420, 421; globosum 87; innominatum 534; ligustici 95, 97; minutum 429, 431; musicola 94; myosotidis

94–97; **namae** 85, **92–94**, 97; **nitidum** 85, **86**, 87, 97; nyctanthidis 94; oxalidis 540; plantagineum 85, 87; plantaginicola 88, 89; **polemonii 84–86**, 97; potentillae 91, 94; punctum 88, 89; pyriforme 94; ranunculi 431; rubrocinctum 94; rhyncosiae 94; selaginellae 94; taraxaci 534, 545; uliginicola 431; ulmariae 94; urticae 431

Syzygospora 677, 683; alba 677

Tachaphantium 822, 838; tiliae 821, 831
Thamnidium 379
Thaxterogaster 720
Thecopsora empetri 602
Thelephora bolaris 391; cinnabarina 391; stereoides 402
Tilletia makutensis 873; **setariae-palmiflorae 873**, 875
Tjibodasia 822; brunnea 827; pezizoides 838
Torula bestae 447; flava 377; herbarum 730; paisii 447
Torulopsis 46, 48; **melibiosum** 44, 48, **49**, 52, 54; **nitratophila** 44, **48**, 52, 54; pinus 48; utilis 455
Trametes 115, 121, 122; epilobii 122; hispida 118, 121; hochnelii 103; inaequalis 122; mollis 120; protracta 107, 108; ribicola 122; serialis 102, 118, 123, 492; squalens 122; stereoides 122; subrosea 106, 121; trabea 107
Trechispora 123; onusta 123
Tremella 677, 691; abietina 878–880; albida 690; aurantia 311; deliquescens 878–880; disciformis 831; fimetaria 831; lacrymalis 879; lutescens 311; palmata 311, 318; palulahuana 690–692
Tricellula 322; inaequalis 320
Trichoderma viride 773
Tricholoma 456, 719–721, 725; subg. Dermoloma 724; atrobrunneum 724; cuneifolium 724; elytroides 724, 725, 727; pes-caprae 721, 722; umbrosum 724, 727
Trichomyces 66
Trichophyton 65, 66, 68, 379; acuminatum 67–69, 71, 73, 78; acutulum 78; areolatum 73, 76, 78; bicolor 70, 78; blanchardi 79; cerebriforme 66, 69, 71, 78, 79; cineraceum 70, 78; circonvolutum 69, 73, 76, 78; crateriforme 67–69, 71, 75, 78, 357; currii, 79; depilans 66, 68, 69, 79; effractum 69, 71, 78; epilans 66, 68, 69, 71, 73, 79; exsiccatum 69, 78; flavum 68–71, 79; floriforme 73, 76–78; fumatum 69, 71, 78; fuscum 70; fuscum [v.?] sulcatum 78; germen 78; inflatum 79; metagrophytes 66–68, 76, 359, 362, 365, 452, 477, 478, 480; ochropyrraceum 70, 78; pilosum 69, 78; plicatile 68, 69, 71, 78; plicans [v.?] fusisporum 68; polygonum 69, 78; regulare 69, 78; rotundum 71, 78; rubrum 76, 359, 360, 362, 452, 477, 478; sabouraudi 68, 70, 71, 78; spongoides 79; sulfuratum 79; sulfureum 69, 71, 73, 75, 78, 358; tonsurans 65, 354; tonsurans v. acuminatum 358; tonsurans v. cerebriforme 358; tonsurans v. crateriforme 79, 358; tonsurans v. sulfureum 71, 79, 358; umbilicatum 69, 71, 78; verrucosum 66, 67
Trichosporon 379
Trichothecium 379; roseum 168, 729
Tromera 865; olivacea 869
Truncatella 733, 768
Tympanis 865, 867, 871; abietis 867, 869, 871; amphiboloides 865; olivacea 869

Umbellula 736
Underwoodia 711, 717; campbellii 714, 715; columnaris 717; sparassoides 714
Uraecium **extensum 607**; lucumae 601
Uredinopsis investita 608
Uredo **acaciae-burseriae 607**; **adenocaulonis 607**; **alaskana 607**; ammophilina 604; antiguensis 604; **arida 607**; bauhiniicola 606; biocellata 606; boutelouae 161; chardonii 144; cherimoliae 604; chloridis-berroi 132; chloridis-polydactylidis 132; **consimilis 607**; costina 605; ctenii 161; **cubensis 607**; cupulata 604; dactyloctenii 158; dactyloçteniicola 157; degener 605; elusine-indicae 149; empetri 602; erythroxylonis 601; gentianae 608; **hoffmanseggiae 608**; inquirenda 607; **investita 608**; jatrophicola 604; lucumae 601; **mexicana 608**; **neopustulata 608**; **obovata 608**; peridermiospora 131; **piscariae 608**; plucheae 606; pustulata 608; sapotae 607; spartinae-strictae 152, 154; **spigeliae 608**

Uromyces 126–128, 152; acuminatus 143, 152–154, 159, 160; acuminatus [race] magnatus 159; acuminatus [race] polemonii 159; archerianus 152, 153, 155, 156; argutus 152–154; beckmanniae 127; brandegei 148; chloridis 155, 156; claytoniae 575, 585, 589; dactyloctenii 152, 153, 156, 157; dactyloctenicola 152, 157; **eugentianae 608**; gentianae 608; **kenyensis** 152, 153, **158**; leptochloae 152, 153, 160; magnatus 159; microchloae 152, 153, 157; phaseoli 20, 22; spartinae 159;

sprague ae 580, 582, 589; trichoneurae 152–154; tripogonicola 154, 155; unitus 580, 581, 584, 589; unitus ssp. **pacificus** 581, **583**–585, 589; unitus ssp. **montanensis** 581, **582,** 584, 585, 589; unitus ssp. **sprague ae** 581, **582,** 584, 585, 589; unitus ssp. **unitus 581,** 584, 585, 589
Ustilago bothriochloae 875; calandriniae 586, 689; calandriniicola 585, 586, 589; claytoniae 586, 589; eriocauli 408; **jagdishwari** 407, **408; nelsoniana 585,** 586, 589; **saubouriana** 873, 875

Venturia 591; dianthi 591, 592; inaequalis 592–595; macularis 592; pirina 593; rosae 591–594
Verticillium 379
Vibrissea 696, 697, 703; foliorum 702–704; lutea 706; truncorum 703, 704

Xanthochrous tamaricis 107
Xanthomonas campestris 801; hederae 801; phaseoli 801; pruni 801; vesicatoria 801
Xenogloea 288, 821; eriophori 288, 838
Xeromphalina 723; tenuipes 723
Xylodon candidum f. lenzitoideum 108

Zaghouania 637
Zygorhizidium melosirae 277; planktonicum 276, 277
Zygorhynchus 379
Zygosaccharomyces 42, 45; pastori 42, 44, 45; pini 41–45

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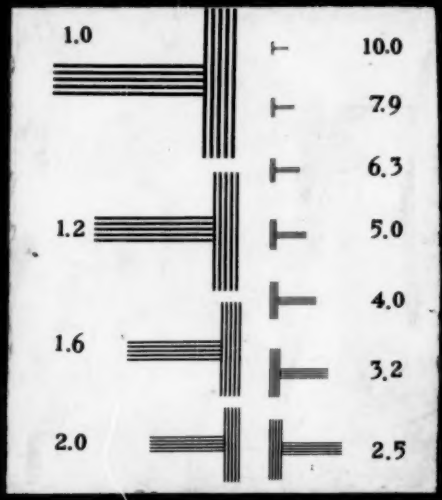

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INSTRUCTIONS Resolution is expressed in terms of the lines per millimeter recorded by a particular film under specified conditions. Numerals in chart indicate the number of lines per millimeter in adjacent "T-shaped" groupings.

In microfilming, it is necessary to determine the reduction ratio and multiply the number of lines in the chart by this value to find the number of lines recorded by the film. As an aid in determining the reduction ratio, the line above is 100 millimeters in length. Measuring this line in the film image and dividing the length into 100 gives the reduction ratio. Example: the line is 20 mm. long in the film image, and 100/20 = 5.

Examine "T-shaped" line groupings in the film with microscope, and note the number adjacent to finest lines recorded sharply and distinctly. Multiply this number by the reduction factor to obtain resolving power in lines per millimeter. Example: 7.9 group of lines is clearly recorded while lines in the 10.0 group are not distinctly separated. Reduction ratio is 5, and 7.9 x 5 = 39.5 lines per millimeter recorded satisfactorily. 10.0 x 5 = 50 lines per millimeter which are not recorded satisfactorily. Under the particular conditions, maximum resolution is between 39.5 and 50 lines per millimeter.

Resolution, as measured on the film, is a test of the entire photographic system, including lens, exposure, processing, and other factors. These rarely utilize maximum resolution of the film. Vibrations during exposure, lack of critical focus, and exposures yielding very dense negatives are to be avoided.